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<b>(54) Title:</b> ELONGATION FACTOR-2 KINASE (EF-2 KINASE) AND METHODS OF USE THEREFOR  <b>(57) Abstract</b>  A new superfamily of protein kinases has been discovered that centers around eukaryotic elongation factor-2 kinase (eEF-2 kinase). The protein kinases of this new superfamily have the following characteristics: 1) sequence similarity to eEF-2 kinase; 2) no sequence similarity to the protein kinases of either the serine/threonine/tyrosine kinase or histidine kinase superfamily; and, 3) specifically phosphorylates $\alpha$ -helical regions of proteins as opposed to $\beta$ -turns, as seen in all other protein kinases. Assays have been developed utilizing eEF-2 kinase and a phosphorylation target consisting of a novel $\alpha$ -helical 16-amino acid peptide sequence to facilitate high-throughput screening for compounds that can specifically inhibit this protein kinase that has been implicated tumor growth and other hyperproliferative disorders. Additionally, the disclosed invention includes assessing eEF-2 kinase levels for diagnostic purposes, and therapeutic formulations to inhibit eEF-2 kinase activity.		

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## ELONGATION FACTOR-2 KINASE (EF-2 KINASE) AND METHODS OF USE THEREFOR

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### FIELD OF THE INVENTION

This invention relates generally to the identification of a new superfamily of eukaryotic protein kinases and the use of one member of this superfamily, elongation factor-2 kinase (eEF-2 kinase), in assays to screen for specific inhibitors. Specifically, this invention provides an isolated nucleic acid encoding a heart protein kinase, a melanoma  
10 protein kinase and a ch4 protein kinase. Specific inhibitors of the eEF-2 kinase may be potent therapeutics for amelioration of malignant transformation. Additionally, sequences complementary to eEF-2 kinase may have therapeutic efficacy as antisense drugs or be used in gene therapy. Specifically, the invention relates to assays developed using the recombinant eEF-2 kinase to screen for inhibitors of phosphorylation of a  
15 peptide derived from the myosin heavy chain (MHC) protein.

### BACKGROUND OF THE INVENTION

Protein phosphorylation plays a critical role in many cellular processes (Krebs (1994) *Trends Biochem. Sci.* 19:439; Hanks and Hunter, (1996) *FASEB J.* 9:576-596; Hardie  
20 and Hanks, (1995) *The Protein Kinase Facts Book* (Academic, London)). There are two well-characterized superfamilies of protein kinases, with most of the protein kinases belonging to the serine/threonine/tyrosine kinase superfamily (Hanks and Hunter, (1996); Hardie and Hanks, (1995)). The characterization of several hundred members of this superfamily revealed that they all share a similar structural  
25 organization of their catalytic domains which consist of twelve conserved subdomains (Hanks and Hunter, (1996); Hardie and Hanks, (1995)). The other superfamily is referred to as the histidine kinase superfamily and is involved in the prokaryotic two-component signal transduction system, acting as sensor components (Stock et al., (1989) *Microbiol. Rev.* 53:450-490; Parkinson and Kofoed, (1992) *Annu. Rev. Genet.*  
30 26:71-112; Swanson, et al., (1994) *Trends Biochem. Sci.* 19:485-490). Recently, eukaryotic members of this superfamily have also been described (Chang et al., (1993) *Science* 263:539-544; Ota and Varshavsky, (1993) *Science* 262:566-569; Maeda et al.,

(1994) *Nature* 369:242-245). Mitochondrial protein kinases have also recently been described that show structural homology to the histidine kinases, but phosphorylate their substrates on serine (Popov et al., (1992) *J. Biol. Chem.* 267:13127-13130; Popov et al., (1993) *J. Biol. Chem.* 268:26602-22606). Finally, several new protein kinases  
5 have been reported that show a lack of homology with either of the kinase superfamilies (Maru and Witte, (1991) *Cell* 67:459-468; Beeler et al., (1994) *Mol. Cell. Biol.* 14:982-988; Dikstein et al., (1996) *Cell* 84:781-790; Futey et al., (1995) *J. Biol. Chem.* 270:523-529; Eichenger et al., (1996) *EMBO J.* 15:5547-5556). However, these protein kinases are viewed as an exception to the general rule as they have yet to  
10 be fully characterized.

The cloning and sequencing of the extensively characterized eukaryotic elongation factor-2 kinase (eEF-2 kinase) from a variety of eukaryotic organisms has now revealed the existence of a novel class of protein kinases (Ryazanov et al., (1997) *Proc. Natl. Acad. Sci., USA* 94:4884-4889). eEF-2 kinase, previously known as  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase III, is highly specific for phosphorylation of elongation factor-2 (eEF-2), an abundant cytoplasmic protein that catalyzes the movement of the ribosome along mRNA during translation in eukaryotic cells (reviewed in Ryazanov and Spirin, (1993) In *Translational Regulation of Gene Expression* (Plenum, New York) Vol. 2, pp. 433-455; Nairn and Palfrey, (1996) In *Translational Control* (CSHL Press, New York) pp. 295-318). All mammalian tissues, and various invertebrate organisms, exhibit eEF-2 kinase activity (Abdelmajid et al., (1993) *Int. J. Dev. Biol.* 37:279-290). eEF-2 kinase catalyzes the phosphorylation of eEF-2 at two highly conserved threonine residues located within a GTP-binding domain (Ryazanov and Spirin, (1993) In *Translational Regulation of Gene Expression* (Plenum, New York) Vol. 2, pp. 433-455; Nairn and Palfrey, (1996) In *Translational Control* (CSHL Press, New York) pp. 295-318). When eEF-2 is phosphorylated, it becomes inactive with respect to protein synthesis (Ryazanov et al., (1988) *Nature* 334:170-173). Since eEF-2 phosphorylation is dependent on  $\text{Ca}^{2+}$  and calmodulin, eEF-2 kinase plays a pivotal  
25  
30 role in modulating the protein synthesis rate in response to changes in intracellular



calcium concentration. Phosphorylation of eEF-2 has also been linked to the regulation of cell cycle progression. For example, transient phosphorylation of eEF-2 occurs during the mitogenic stimulation of quiescent cells (Palfrey et al., (1987) *J. Biol. Chem.* 262:9785-9792) and during mitosis (Celis et al., (1990) *Proc. Natl. Acad. Sci., USA* 87:4231-4235). In addition, changes in the level of eEF-2 kinase activity is associated with a host of cellular processes such as cellular differentiation (End et al., (1982) *J. Biol. Chem.* 257:9223-9225; Koizumi et al., (1989) *FEBS Lett.* 253:55-58; Brady et al., (1990) *J. Neurochem.* 54:1034-1039), oogenesis (Severinov et al., (1990) *New Biol.* 2: 887-893), and malignant transformation (Bagaglio et al., (1993) *Cancer Res.* 53:2260-2264).

The sequence eEF-2 kinase appears to have no homology to either the  $Ca^{2+}$ /calmodulin-dependent protein kinases or to any members of the known protein kinase superfamilies (Ryazanov et al., (1997) *Proc. Natl. Acad. Sci., USA* 94:4884-4889). However, the recently described myosin heavy chain kinase A (MHCK A) from *Dictyostelium* (Futey et al., (1995) *J. Biol. Chem.* 270:523-529) shows a great deal of homology with eEF-2 kinase. These two kinases define a novel class of protein kinases that may represent a new superfamily.

Evidence for MHCK and eEF-2 kinase forming the core of a new superfamily is as follows. MHCK A from *Dictyostelium*, has a demonstrated role in the regulation of myosin assembly (Futey et al., (1995) *J. Biol. Chem.* 270:523-529; Côté et al., (1997) *J. Biol. Chem.* 272:6846-6849). eEF-2 kinase is a ubiquitous  $Ca^{2+}$ /calmodulin-dependant protein kinase involved in the regulation of protein synthesis by  $Ca^{2+}$  (Redpath et al., (1996) *J. Biol. Chem.* 271:17547-17554; Ryazanov et al., (1997) *Proc. Natl. Acad. Sci., USA* 94:4884-4889). Both MHCK A and eEF-2 kinase display no homology to any of the known protein kinases, but are strikingly similar to each other; amino acid sequences of their catalytic domains are 40% identical. Another protein kinase homologous to MHCK A and eEF-2 kinase has recently been identified in *Dictyostelium* (Clancy et al., (1997) *J. Biol. Chem.* 272:11812-11815), and an

expressed sequence tag (EST) sequence, with a high degree of similarity to the catalytic domain common to both MHCK A and eEF-2 kinase, has been deposited in GenBank (clone FC-AN09/accession #C22986). An amino acid sequence alignment of the catalytic domains of these new protein kinases is shown in Figure 1A. These kinases  
5 have a catalytic domain of approximately 200 amino acids which can be subdivided into seven conserved subdomains. Subdomains V, VI, and VII have a predicted  $\beta$ -sheet structure and are presumably involved in ATP-binding, while subdomains I through IV may be involved in substrate binding and catalysis. These new protein kinases have no homology to the members of the eukaryotic serine/threonine/tyrosine protein kinase  
10 superfamily with the exception of the GXGXXG motif in subdomain VI which is present in many ATP-binding proteins. Thus, MHCK A, eEF-2 kinase, and related protein kinases may represent a new superfamily. Evolutionary analysis of these new kinases (Figure 1B) reveals that they can be subdivided into 2 families: the eEF-2 kinase family which includes eEF-2 kinases from different organisms, and the MHCK  
15 family which includes MHCK A, MHCK B and FC-AN09. These two families appear to have split more than a billion years ago.

An interesting question is why does nature employ these unusual kinases to phosphorylate eEF-2 and myosin heavy chains? Perhaps the answer is related to the  
20 secondary structure of the phosphorylation sites. As was originally reported by Small et al. (Small et al., (1977), *Biochim. Biophys. Res. Comm.* 79:341-346), phosphorylation sites are usually located at predicted  $\beta$ -turns. Subsequent studies, including X-ray crystallographic data, demonstrated that phosphoacceptor sites in substrates of conventional protein kinases are often located in turns or loops and usually  
25 have flexible extended conformation (Knighton et al., (1991) *Science* 253:414-420; Pinna and Ruzzene (1996) *Biochim. Biophys. Acta* 1314:191-225). In contrast to this, the existing evidence suggests that the peptides around phosphorylation sites for eEF-2 kinases and MHCK A have an  $\alpha$ -helical conformation. The two major phosphorylation sites for MHCK A are located in a region which has a coiled-coil  $\alpha$ -helical structure  
30 (Vaillancourt et al., (1988) *J. Biol. Chem.* 253:10082-10087). The major

phosphorylation site in eEF-2, threonine 56, is located within a sequence which is homologous among all translational elongation factors. In the crystal structure of the prokaryotic elongation factor EF-Tu, this sequence has an  $\alpha$ -helical conformation (Polekhina *et al.*, (1996) *Structure* 4:1141-1151; Abel *et al.*, (1996) *Structure* 4:1153-  
5 1159). These facts suggest that eEF-2 kinase and MHCK A differ from conventional protein kinases in that they phosphorylate amino acids located within  $\alpha$ -helices.

Thus, in addition to the two well-characterized superfamily of eukaryotic protein kinases, which phosphorylate amino acids located in loops and turns, there appears to  
10 be a third superfamily of  $\alpha$ -helix-directed kinases.

#### SUMMARY OF THE INVENTION

In accordance with the present invention, a new superfamily of protein kinases and corresponding methods for assaying their phosphorylation activity are disclosed. The  
15 protein kinases of this new superfamily have the following characteristics: 1) No significant sequence homology to protein kinases of either the serine/threonine/tyrosine kinase or histidine kinase super families; 2) moderate to high ( $\geq 40\%$ ) to eEF-2 kinases from any organism; and, 3) phosphorylates an amino acid within an  $\alpha$ -helical domain.

20 The present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes eEF-2 kinase; preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the eEF-2 kinase has a nucleotide sequence or is complementary to a DNA sequence shown in Figure 5 (SEQ ID NO: 1, 3, and 9).

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The present invention also relates to an isolated nucleic acid molecule which encodes a heart protein kinase. The present invention also relates to an isolated nucleic acid molecule which encodes a melanoma protein kinase. The present invention also relates to an isolated nucleic acid molecule which encodes a ch4 protein kinase.

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The human and murine DNA sequences of the eEF-2 kinase gene of the present invention or portions thereof, may be prepared as probes to screen for complementary sequences and genomic clones in the same or alternate species. The present invention extends to probes so prepared that may be provided for screening cDNA and genomic  
5 libraries for the eEF-2 kinase gene.

The present invention also includes eEF-2 kinase proteins having the activities noted herein, and that display the amino acid sequences set forth and described above and selected from SEQ ID NO: 2, 4, and 10.

10

The invention includes an assay system for screening of potential drugs effective at attenuating eEF-2 kinase activity of target mammalian cells by interrupting or potentiating the phosphorylation of eEF-2. It is a further object of the present invention to provide antibodies to the phosphorylated eEF-2 kinase target, and methods for their  
15 preparation, including recombinant means.

It is a further object of the present invention to provide a method for detecting eEF-2 kinase activity in mammals in which invasive, spontaneous, or idiopathic pathological states are suspected to be present.

20

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of eEF-2 kinase, so as to alter the adverse consequences of such presence or activity, or where beneficial, to enhance such activity.

25

It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of eEF-2 kinase, so as to treat or avert the adverse consequences of invasive, spontaneous or idiopathic pathological states.

It is a still further object of the present invention to provide pharmaceutical compositions for use in therapeutic methods which comprise or are based upon a sequence complementary to that of the eEF-2 kinase mRNA, which would form the basis for an antisense therapeutic that can reduce expression, and thus activity, of eEF-  
5 2 kinase.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B.

10 (A) Sequence alignment of the catalytic domains of human eEF-2 kinase, *C. elegans* eEF-2 kinase, MHCK A, MHCK B and clone FC-ANO9. Identical amino acids (bold) and conserved hydrophobic amino acids ("") are noted. (B). Phylogenetic tree of sequences shown in (A), with the addition of mouse and rat eEF-2 kinases. Tree was obtained using the J. Hein method with PAM250 residue weight table. The  
15 following accession numbers were used for the sequences: U93846-U93850, 1495779, 1170675, 1903458, C22986.

Figure 2. Expression of recombinant eEF-2 kinase *in vitro*. Plasmid DNA from clones *Cefk-1*, *Cefk-2*, as well as mouse and human eEF-2 kinase cDNA  
20 were used in the TNT wheat germ extract coupled transcription/translation system (Promega). [<sup>35</sup>S]Methionine-labeled products were then analyzed by SDS/PAGE.

Figure 3. Activity of recombinant eEF-2 kinase *in vitro*. A large scale (0.5 ml)  
25 reaction using a mixture of *Cefk-1* and *Cefk-2* plasmids was run as in Figure 2, with the omission of labeled methionine. In the control experiment, the reaction was run with a plasmid containing a luciferase gene. (A) The reaction mixtures were separated by chromatography on a Mono Q column as described. (B) eEF-2 kinase activity in fractions  
30 was measured as the ability to phosphorylate purified rabbit eEF-2 in the

presence of [ $\gamma$ - $^{32}$ P]ATP. Purified rabbit reticulocyte eEF-2 kinase was used in the (+) control experiments. (C)  $\text{Ca}^{2+}$ /calmodulin-dependency of recombinant *C. elegans* eEF-2 kinase. Mono Q fraction 25 was assayed in a standard eEF-2 kinase assay in the presence and absence of  $\text{Ca}^{2+}$  and calmodulin and 20  $\mu\text{M}$  trifluoperazine (TFP) or *N*-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide (W7). (D)  $\text{Ca}^{2+}$ /calmodulin-dependency of recombinant human eEF-2 kinase. Human eEF-2 kinase cDNA was expressed in a coupled transcription/translation system as described above and eEF-2 kinase activity was assayed without further purification.

Figure 4. Northern blot analysis of tissue distribution of mouse eEF-2 kinase mRNA. Northern blots of mouse tissue containing 2  $\mu\text{g}$  of polyadenylated RNA per lane were probed with the random-primed  $^{32}\text{P}$ -labeled mouse eEF-2 kinase cDNA (31). The major transcript appeared at 3.1 kb and minor transcripts at 6.1 and 2.5 kb were also apparent (exposure time, 5 days). The same blots were stripped and rehybridized with a human eEF-2 cDNA (exposure time, 4 days).

Figure 5. Sequence alignment of *C. elegans*, mouse, human eEF-2 kinase, and the catalytic domain of *Dictyostelium discoideum* MHCK A. Identical amino acids are indicated by dark blue boxed regions and chemically conserved amino acids are indicated by light blue shaded regions. Amino acids in the human sequence that are identical to the mouse sequence are represented by dots. Amino acids underlined in black correspond to the six regions that match peptides obtained from the sequencing of purified rabbit reticulocyte eEF-2 kinase. The GXGXXG nucleotide-binding motif is underlined in red. The blue dashed line over residues 625-632 in *C. elegans* eEF-2 kinases designates the amino acids corresponding to exon 4, which is missing in *Cefk-2*.

- Figure 6. Substrate specificity of eEF-2 kinase and MHCK A. Phosphorylation assays containing eEF-2 kinase (~50 ng) or MHCK A (0.2  $\mu$ g) and either 0.5  $\mu$ g rabbit reticulocyte eEF-2 or 0.1  $\mu$ g *Dictyostelium* myosin were performed under standard conditions except that incubation time was extended to 10 min.
- Figure 7. Schematic representation of the structure of mammalian and *C. elegans* eEF-2 kinases and MHCK A. The homologous regions are represented by dark shading. The regions of weak similarity are represented by light shading. The position of the GXGXXG motif is indicated by vertical arrows.
- Figure 8. Assay for eEF-2 kinase activity. Recombinant eEF-2 kinase (2  $\mu$ g) was incubated with increasing concentrations of a peptide phosphorylation target (RKKGESEKTKTKEFL) in a buffer consisting of 12.5 mM Hepes-KOH (pH 7.4), 2.5 mM magnesium acetate, 1.25 mM DTT, 25  $\mu$ M CaCl<sub>2</sub>, 0.5  $\mu$ g calmodulin, 100  $\mu$ M ATP, and 0.5  $\mu$ Ci [ $\gamma$ -<sup>33</sup>P]ATP in a total volume of 50  $\mu$ l. Samples were incubated at 30°C and aliquots were withdrawn at various time points, and the reaction was terminated by incubation in an ice water bath. The aliquots were then spotted onto phosphocellulose paper (2 cm x 2 cm) and washed (4 x 4 min) with 75 mM phosphoric acid. The papers were then rinsed with 100% ethanol, dried, and then counted in a scintillation counter.
- Figure 9. Sequence alignment of *C. elegans*, mouse, human eEF-2 kinase, and the catalytic domain of *Dictyostelium discoideum* MHCK A, heart kinase, melanoma kinase and ch4 kinase. Identical amino acids are indicated by dark blue boxed regions and chemically conserved amino acids are indicated by light blue shaded regions.



- Figure 10. pH-dependence of eEF-2 kinase activity: eEF-2 kinase activity was assayed by phosphorylation of the peptide substrate, MH-I at 0.2 pH unit intervals from pH 6.0-8.0.
- 5 Figure 11. Dependence of protein synthesis on intracellular pH: Protein synthesis was measured by  $^3\text{H}$ -Leu-pulse incorporation in GH3 cells at various pH. Actual intracellular pH was determined by fluorimetric analysis.
- 10 Figure 12. Cytotoxicity assays of clones overexpressing eEF-2 kinase. MTT assays were done in quadruplicate and the results are presented as mean of values obtained.
- 15 Figure 13. Enzymatic activity of recombinant forms of human eEF-2 kinase expressed in *E. coli*. Autoradiograph of kinase assays with recombinant 6xHis-tagged eEF-2 kinase (lanes 1-4)- and GST-tagged-eEF-2 kinase (lanes 5-8). Reactions were done with and without eEF-2 (0.5  $\mu\text{g}$ ), and with and without calmodulin (0.5  $\mu\text{g}$ ). In autophosphorylation assays with 6xHis-eEF-2 kinase, 5  $\mu\text{g}$  of eEF-2 kinase were used.
- 20 Figure 14. Enzymatic activity of eEF-2 kinase deletion mutants. Autoradiograph of eEF-2 kinase assay using total bacterial lysates of eEF-2 kinase deletion mutants. eEF-2 kinase assay was performed as described in text using 1  $\mu\text{g}$  of total protein, and reactions were analyzed by 8% SDS-PAGE. Labels above lanes designate which amino acids are
- 25 deleted in the mutant being assayed. Deletion of amino acids 51-335 causes loss of autophosphorylation activity, and a loss of eEF-2 kinase activity. Deletion of amino acids 521-725 also causes a loss of eEF-2 kinase activity.
- 30 Figure 15. Schematic representation of wild-type and mutant eEF-2 kinases. Hatched areas represent the putative catalytic domain. Black-shaded

areas represent regions conserved between various eEF-2 kinases. White-shaded areas represent nonconserved regions. Numbers at the top of the schematic represent the amino acids at the boundaries of each of these regions. eEF-2 kinase activity and autophosphorylation activity of each mutant are summarized on the right with (+) representing presence of activity and (-) representing absence of activity. n/d = not determined

#### Figures 16A-16B

(A) Phosphorylation of synthetic peptides by eEF-2 kinase. See text for experimental details. X-axis = time in minutes. Y-axis =  $^{33}\text{P}$  incorporation into peptides in counts per minute. (B) Sequences of peptides corresponding to phosphorylation site in *Dictyostelium* myosin heavy chains (MHC, MH-1) and eEF-2. Phosphoacceptor threonines are designated by an asterisk. Helical wheel representation of both peptides is also shown. Phosphoacceptor threonines are circled.

#### DETAILED DESCRIPTION

Novel protein kinase inhibitors have the potential to form the basis for pharmaceutical compositions that can ameliorate malignant transformation. In order to find these inhibitors, libraries of chemical compounds are routinely screened using an automated protein kinase assay. The drawback to this approach is that most protein kinases have a very similar structure, thus making it difficult to specific inhibitors which act solely on a particular protein kinase. It was determined the primary structure of eEF-2 kinase, a ubiquitous enzyme which is involved in the regulation of protein synthesis and the cell cycle. Unexpectedly, it was found that eEF-2 kinase has a unique structure. It has no homology to any other mammalian protein kinase. This feature makes eEF-2 kinase an ideal target in the search for a specific protein kinase inhibitor. Since preliminary evidence suggests that eEF-2 kinase is upregulated in human cancers, including, but not limited to, breast cancer, identification of specific inhibitors of eEF-2 kinase can eventually lead to the development of novel anticancer drugs. In order be

able to perform a high throughput screen for an eEF-2 kinase inhibitor, it is first necessary to develop a simple assay which is amenable to automation. The existing assay involves incubation of partially purified eEF-2 kinase along with purified eEF-2 and [ $\gamma$ - $^{32}$ P]ATP as substrates in the presence of increasing concentrations of candidate inhibitors. Results are then obtained by electrophoretic separation of the reaction mixtures, followed by autoradiography. Results are then quantified by either densitometry or scintillation counting of excised bands from the gel containing  $^{32}$ P-eEF-2. Clearly, this assay, as it stands, is time-consuming, expensive, and not amenable to automation. Furthermore, it is difficult to purify large amounts of native eEF-2 required to perform multiple assays, and attempts to overexpress a recombinant form of eEF-2 were unsuccessful as its overexpression was toxic to host strains (personal communication from James Bodley, University of Minnesota, Minneapolis). Therefore, new methodologies for determining eEF-2 kinase activity were developed, which involves the use of a specific peptide substrate; easily and economically manufactured in large scale. These methods are relatively inexpensive, fast, and can be fully automated.

In the first attempt to use a peptide as an eEF-2 kinase substrate, peptides centered around the phosphorylation site of eEF-2 were generated. This strategy did not yield a peptide that was functional in phosphorylation assays. Surprisingly, it was found that a 16' mer peptide (RKKFGGESEKTKTKEFL (SEQ ID NO: 20)), based on the phosphorylation site of *Dictyostelium discoideum* MHC, was an acceptable substrate for use with eEF-2 kinase in phosphorylation assays. It is interesting to note that while eEF-2 kinase can phosphorylate a peptide derived from MHC, it is not able to phosphorylate native MHC (Ryazanov et al., (1997) *Proc. Natl. Acad. Sci., USA* 94:4884-4889).

In accordance with the present invention, a new superfamily of protein kinases and corresponding methods for assaying their phosphorylation activity are disclosed. The protein kinases of this new superfamily have the following characteristics: 1) No

significant sequence homology to protein kinases of either the serine/threonine/tyrosine kinase or histidine kinase super families; 2) moderate to high ( $\geq 40\%$ ) to eEF-2 kinases from any organism; and, 3) phosphorylates an amino acid within an  $\alpha$ -helical domain.

- 5 The present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes eEF-2 kinase; preferably a nucleic acid molecule, in particular a recombinant DNA molecule or cloned gene, encoding the eEF-2 kinase has a nucleotide sequence or is complementary to a DNA sequence shown in Figure 5 (SEQ ID NO: 1, 3, and 9).

10

The human and murine DNA sequences of the eEF-2 kinase gene of the present invention or portions thereof, may be prepared as probes to screen for complementary sequences and genomic clones in the same or alternate species. The present invention extends to probes so prepared that may be provided for screening cDNA and genomic  
15 libraries for the eEF-2 kinase gene. For example, the probes may be prepared with a variety of known vectors, such as the phage  $\lambda$  vector. The present invention also includes the preparation of plasmids including such vectors, and the use of the DNA sequences to construct vectors expressing antisense RNA or ribozymes which would attack the mRNAs of any or all of the DNA sequences set forth in FIGURES 5 (SEQ  
20 ID NO: 1, 3, and 9). Correspondingly, the preparation of antisense RNA and ribozymes are included herein.

The present invention also includes eEF-2 kinase proteins having the activities noted herein, and that display the amino acid sequences set forth and described above and  
25 selected from SEQ ID NO: 2, 4, and 10.

This invention provides an isolated nucleic acid which encodes a heart protein kinase, including analogs, fragments, variants, and mutants, thereof. In one embodiment the nucleic acid has a nucleotide sequence having at least 90% similarity with the nucleic  
30 acid coding sequence as shown in Figure 9. In one embodiment the nucleic acid has a

nucleotide sequence having at least 85% similarity with the nucleic acid coding sequence as shown in Figure 9. In one embodiment the nucleic acid has a nucleotide sequence having at least 80% similarity with the nucleic acid coding sequence as shown in Figure 9. In another embodiment the nucleic acid has the following sequence:

5 CACTTGACTGCAGGGATAAAGAAGAAAATTCTATCCAGGGTCGC  
AGCCCTGAGACTGAGGCTGGAGGAAAAGGAAAATTCGAGGAAG  
AACTCCATCGTGAAGAAGACACCTAAGTTTGAAAGGTCCTTATC  
CCGCACTGATGAGAAAAGAGACCCCAAAAAGGGCCCCTTGCAAA  
GCTGAAGGGGAAAGCTCCAGTATTGCTGAAGAGGATCCAGGCCGA  
10 GATGGCTCCCGAGCACTCCGGAAATATAAAGTTGAGCTGCCAGTTT TCAGA  
AATCCATGAAGACTCTACCGTCTGCTGGACAAAAGATTCCAAGTCGATAG  
CCCAGGCCAAGAAAAGCGCAGGGGACAACCTCCAGTGTTTCCTTGGCCATC  
GTCCAAGCTGGTCAGAAGGACCAGGGCCTGTATTACTGCTGCCTCAAGAA  
CAGTTATGGAAAAGTCACTGCTGAGTTTAACCTCACAGCTGAAGTTCTCAA  
15 ACAGCTTTCAAGTCACCAGAATACTAGAGGATGTGAAGAGATTGAATTCA  
GCCAGCTCATCTTCAAAGAAGATGTTTTCAATGACAGCTACTTCGGGGAC  
CACCTACGTGGCCAGATCTCCACGGAGGAGCTTCACTTTGGCGAAGGGGT  
GCACCGCAAAGCTTTCCGGAGCAAGGTGATGCAGGGCCTCATGCCGGTCT  
TCCAGCCCCGGCCACGCATGCGTACTCAAGGTGCACAATGCCGTCGCCCAT  
20 GGGACCAGAAACAATGACGAACTTGTGCAGAGGAACTACAAACTGGCTG  
CCCAGGAATGCTACGTGCAGAAATACTGCCAGATACTACGCCAAGATCTAC  
GCCGCTGAAGCACAGCCTCTGGAAGGCTTCGGAGAGGTGCCGGAGATCAT  
TCCTATTTTCCTTATCCATCGGCCCCGAGAACAAACATCCCATATGCCACAGT  
GGAAGAAGAGCTGATTGGAGAATTCGTGAAGTATTCCATCCGGGACGGGA  
25 AGGAAATCAACTTCCTCAGACGAGATTCAGAGGCTGGCCAGAAATGTTGC  
ACCTTCCAGCACTGGGTATACCAGAAAACAAGTGGCTGTCTCCTGGTSAC  
GGACATGCAGGGTGTGGGAATGAAGTTAACTGACGTTGGCATAGCAACAC  
TAGCTAGAGGGTACAAAGGATTTAAGGGCAACTGTTCCATGACCTTCATT  
GATCAGTTCAGAGCGCTGCATCAGTGTAACAAGTACTGTAAAATGCTGGG  
30 GCTGAAATCCCTTCAAACAACAGCCAGAAGCCCAGGAAGCCCATCGTCG

GGAAAGGCAGGGTTCCGACAAACGCCACGCAGGTGAAGACGCCTGAGTC  
TGAGACGCCGCCCCGCAGAAAGAAAAACCTAGCCTCCCTCCTCCCTTCATC  
ACCAGTGACCACCAAGCCAGCATCGCGCAGGCTTGCGCGTGGACATCTGC  
AAGCACACAAGGGACACGAGCCTGCAGCCTGCAGCCGAGTGCCAGTCCTC  
5 TCAGCTCCTATCACTGGCTGTCTGCTGAAATGACAATGGCATGGCTCTTCC  
AGACTAGCCTTGTAGAGAGACTTAGCAGTTCTGTTGATGCTCTCAAAGGC  
AGCCCACTGTTTGTGTACACAGCTAGCCTTTCTACACACACCCTCCCCTCC  
CACCGCATCGTCTATCTATCTGTGTGTCGCGCGTGGTTTGTTGACAAGAGT  
TCCCCCGCTGCCTTGGCGACTGGCCACTGTCAAAATCCTTCCCACCTCGAC  
10 CCCCTCACCTCAGGATGTTCTGTCAGTCATGAATGTCAAGTTGTTGTTATC  
AGTGTCACCGACGCTATTGTTGCTGGAGGCGGCTTCCCAGATGCGAGCCC  
ATTTCCCGCCACTACCCACGCAGCCTGGCACAGTGTTCTGTTTCATTAAAT  
TCATATTTAAGCAAAAAAAAAAAAAAAAAAAAAA

- 15 This invention provides a heart protein kinase which has the amino acid sequence as follows:

HLTAGIKKKILSRVAALRLRLEEKENSRKNSIVKKTPKFERSLSRTDEKRDPKR  
APCKAEGKAPVLLKRIQAEMAPEHSGNIKLSCQFSEIHEDSTVCWTKDSKSIA  
QAKKSAGDNSSVSLAIVQAGQKDQGLYYCCLKNSYGKVTAEFNLTAEVLKQ  
20 LSSHQNTRGCEEIEFSQLIFKEDVFND SYFGDHLRGQISTEELHFGEGVHRKAF  
RSKVMQGLMPVFQPGHACVLKVHNAVAHGTRNNDLVQRNYKLAAQECY  
VQNTARYYAKIYAAEAQPLEGFGEVPEIPIFLIHRPENNIPYATVEEELIGEFV  
KYSIRDGKEINFLRRDSEAGQKCCTFQHWVYQKTSGCLLVTD MQGVGMKLT  
DVGIATLARGYKGFKGNCSMTFIDQFRALHQC NKYCKMLGLKSLQNNSQKP  
25 RKPIVGKGRVPTNATQVKTPESETPPAERKT

- This invention provides an isolated nucleic acid which encodes a melanoma protein kinase, including analogs, fragments, variants, and mutants, thereof. In one embodiment the nucleic acid has a nucleotide sequence having at least 90% similarity with the nucleic acid coding sequence as shown in Figure 9. In one embodiment the nucleic acid has a
- 30



nucleotide sequence having at least 85% similarity with the nucleic acid coding sequence as shown in Figure 9. In one embodiment the nucleic acid has a nucleotide sequence having at least 80% similarity with the nucleic acid coding sequence as shown in Figure 9. On another embodiment the melanoma kinase has the following sequence:

5 GGCACGAGCTCGTCCACATATTACTATTATTCAGCTGTGGAAAGAAATAAC  
CTGATGAGGTTGTCACAGAGTATTCCCTTCGTTCTGTACCTCCACGAGGCG  
GCCTGTCACAGTGTCCCGTCTGGAGGAGAGTTCTCCCAGTATACTGAATA  
ACAGCATGTCTTCATGGTCTCAGCTAGGCCTCTGTGCCAAAATTGAGTTTT  
TAAGTAAAGAGGAAATGGGAGGCGGTTTACGAAGAGCAGTCAAAGTGCT  
10 GTGTACCTGGTCAGAGCACGATATCCTGAAGTCAGGGGCATCTCTATATCAT  
TAAGTCATTTCTTCCTGAGGTGATAAACACATGGTCAAGCATTTATAAAGA  
AGATACGGTTCTACATCTCTGTCTCAGAGAAATACAACAACAGAGAGCAG  
CACAAAAGCTCACATTTGCCTTTAATCAGATGAAACCCAAATCCATACCA  
TATTCTCCAAGGTTCTTGAAGTTTTCTGTTGTACTGCCATTCAGCAGGG  
15 CAGTGGTTCGCTGTAGAAGAGTGCATGACTGGTGAATTTAGAAAATACAA  
CAACAATAATGGTGATGAAATCATTCCTACAAATACTCTAGAAGAGATCA  
TGCTAGCCTTTAGCCACTGGACCTATGAATATAACCAGAGGGGGAGTTACTG  
GTAAGTACTTACAAGGAGTGGGAGAAAACCTTGACTGACCCATCTGTAAT  
AAAAGCTGAAGAAAAAAGATCCTGTGACATGGTTTTTTGGCCCTGCCAATC  
20 TAGGAGAAGATGCAATAAAAAAATTCAGAGCCAAACATCACTGTAATTCT  
TGCTGTCGAAAGCTTAAACTTCCAGATTTGAAGAGGAATGACTACACGCC  
TGATAAAATTATATTTCTCAGGATGAGTCATCAGATTTGAATCTTCAATC  
TGGAATTCCACCAAAGAATCAGAAGCAACAAATTCTGTTCGTCTGATGTTA

25 This invention provides a melanoma kinase protein which has the sequence as follows:  
GTSSSTYYYS AVERNLMRLS QSI PFV PVP PRG EPV TVSR LEES SP SIL NNSM  
SSWSQLGLCAKIEFLSKEEMGGGLRRV KVLCTWSEHDILKSGHLYI IKSFLPE  
VINTWSSIYKEDTVLHLCLREIQQQRAAQKLTF AFNQM KP KSIPYSPRFLEVFL  
LYCHSAGQWF AVEECMTGEFRKYNNNNGDEI IPTNTLEEIMLAFSHWTYEYT  
30 RGELLVLDLQGVGENLTDPSVIKAE EKRS CDMVF GPANLGEDAIKNFRAKHHC



NSCCRKLKLPDLKRNDYTPDKIIFPQDESSDLNLQSGNSTKESEATNSVRLML

This invention provides an isolated nucleic acid which encodes a ch4 protein kinase, including analogs, fragments, variants, and mutants, thereof. In one embodiment the nucleic acid has a nucleotide sequence having at least 90% similarity with the nucleic acid coding sequence as shown in Figure 9. In one embodiment the nucleic acid has a nucleotide sequence having at least 85% similarity with the nucleic acid coding sequence as shown in Figure 9. In one embodiment the nucleic acid has a nucleotide sequence having at least 80% similarity with the nucleic acid coding sequence as shown in Figure 9. In another embodiment the ch 4 kinase has a nucleic acid which encodes the following amino acid sequence:

MCRKRTRARTSAAEASLRASILARDCAAAAIVFLVDRFLYGLDVSGKLLQV  
KGLHKLQPATPIAPQVVIRQARISVNSGKLLKAEYILSSLISNNGATGTWLYRN  
ESDKVLVQSVCIQIRGQILQKLGMWYEAELIWASIVGYLALPQPDKKGLSTS  
15 LGILADIFVSMKNDYEKFKNNPQINLLSLLKEFDHHLLSAAEACKLAAAFSA  
YTPLFVLTAVNIRGTCLLSYSSSNDCPPELKNLHLCEAKEAFEIGLLTKRDDEP  
VTGKQELHSFVKAAFGLTTVHRRLHGETGTVHAASQLCKEAMGKLYNFSTSSR  
SQDREALSQEVMSVIAQVKEHLQVQSFSNVDDRSYVPESFECRLDKLILHGQGDF  
QKILDTYSQHHTSVCEVFESDCGNNKNEQKDAKTGVCITALKTEIKNIDTVST  
20 TQEKPHCQRDTGISSSLMGKNVQRELRRGGRNWNTHSDAFRVSLDQDVETET  
EPSDYSNGEGAVFNKSLSGSQTSSAWSNLSGFSSSASWEEVNYHVDDRSARK  
EPGKEHLVDTQCSTALSEELENDREGRAMHSLHSQLHDLSLQEPNNDNLEPS  
QNQPQQQMPLTPFSPHNTPGIFLAPGAGLLEGAPEGIQEVRNMGPRNTSAHSR  
PSYRSASWSSDSGRPKNMGTHPSVQKEEAFEIIVEFPETNCDVKDRQGKEQGE  
25 EISERGAGPTFKASPSWVDPEGETAESTEDAPLDFHRVLHNSLGNISMLPCSSF  
TPNWPVQNPDSRKSGGPVAEQGIDPDASTVDEEGQLLDSMDVPCTNGHGSH  
RLCILRQPPGQRAETPNSSVSGNILFPVLSEDCTTTEEGNQPGNMLNCSQNSSS  
SSVWWLKSPAFSSGSSEGDSPWSYLNSSGSSWVSLPGKMRKEILEARTLQPD  
DFEKLLAGVRHDWLFQRLNTGVFKPSQLHRAHSLLLKYSKKSELWTAQETI  
30 VYLG DYLT VKKKGRQRNAFWVHHLHQEEILGRYVGKDYKEQKGLWHHFTD

VERQMTAQHYVTEFNKRLYEQNIPTQIFYIPSTILLILEDKTIKGCISVEPYILGE  
FVKLSNNTKVVKTEYKATEYGLAYGHFSYEFSNHRDVVVDLQGWVTGNGK  
GLIYLTDPQIHSVDQKVFTTNFGKRGIFYFFNNQHVECNEICHRLSLTRPSMEKPX

- 5 In a further embodiment of the invention, the full DNA sequence of the recombinant DNA molecule or cloned gene so determined may be operatively linked to an expression control sequence which may be introduced into an appropriate host. The invention accordingly extends to unicellular hosts transformed with the cloned gene or recombinant DNA molecule comprising a DNA sequence encoding eEF-2 kinase, and  
10 more particularly, the complete DNA sequence determined from the sequences set forth above and in SEQ ID NO: 1, 3, and 9.

According to other preferred features of certain preferred embodiments of the present invention, a recombinant expression system is provided to produce biologically active  
15 animal or human eEF-2 kinase.

The present invention naturally contemplates several means for preparation of eEF-2 kinase, including as illustrated herein known recombinant techniques, and the invention is accordingly intended to cover such synthetic preparations within its scope. The  
20 isolation of the cDNA and amino acid sequences disclosed herein facilitates the production of eEF-2 kinase by such recombinant techniques, and accordingly, the invention extends to expression vectors prepared from the disclosed DNA sequences for expression in host systems by recombinant DNA techniques, and to the resulting transformed hosts.

25

The invention includes an assay system for screening of potential drugs effective at attenuating eEF-2 kinase activity of target mammalian cells by interrupting or potentiating the phosphorylation of eEF-2. In one instance, the test drug could be administered to a cellular sample along with ATP carrying a detectable label on its  $\gamma$ -  
30 phosphate that gets transferred to eEF-2, or a peptide substrate, by eEF-2 kinase.

Quantification of the labeled eEF-2 or peptide substrate is diagnostic of the candidate drug's efficacy. A further embodiment would provide for the assay to be performed using a purely *in vitro* system comprised of eEF-2 kinase, ATP or labeled ATP, eEF-2 or peptide analog of a portion of eEF-2 or MHC, appropriate buffer, and detection  
5 reagents and/or instrumentation to detect and quantify the extent of eEF-2 kinase-directed phosphorylation activity..

The assay system could more importantly be adapted to identify drugs or other entities that are capable of binding to the eEF-2 kinase and/or its cognate phosphorylation  
10 target (e.g. eEF-2), either in the cytoplasm or in the nucleus, thereby inhibiting or potentiating eEF-2 kinase activity and its resultant phenotypic outcome. Such an assay would be useful in the development of drugs that would be specific against particular cellular activity, or that would potentiate such activity, in time or in level of activity. For example, such drugs might be used to treat various carcinomas or other  
15 hyperproliferative pathologies.

The present invention likewise extends to antibodies against specifically phosphorylated eEF-2 kinase targets (e.g. eEF-2 or peptide), including naturally raised and recombinantly prepared antibodies. These antibodies and their labeled counterparts are  
20 included within the scope of the present invention for their particular ability in detecting eEF-2 kinase activity *via* detection of the phosphorylated product by ELISA or any other immunoassay known to the skilled artisan.

In the instance where a radioactive label, such as the isotopes  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  
25  $^{36}\text{Cl}$ ,  $^{51}\text{Cr}$ ,  $^{57}\text{Co}$ ,  $^{58}\text{Co}$ ,  $^{59}\text{Fe}$ ,  $^{90}\text{Y}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ , and  $^{186}\text{Re}$  are used, known currently available counting procedures may be utilized. In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques known in the art.

In a further embodiment, the present invention contemplates antagonists of the activity of eEF-2 kinase. In particular, an agent or molecule that inhibits phosphorylation of eEF-2. In a specific embodiment, the antagonist can be a peptide comprising sequences, or sequence variants adjacent to, and including, the phosphorylation site in  
5 either eEF-2 or MHC. It is anticipated that these peptides would be competitive inhibitors of eEF-2 kinase's cognate target.

In still a further embodiment, the invention contemplates antisense drugs such that sequences complementary to the eEF-2 kinase mRNA inhibit production of functional eEF-2 kinase. In a specific embodiment, the antisense drug may be a complementary  
10 oligonucleotide (DNA, RNA, or hybrid thereof), which may or may not be modified so as to have the following characteristics: 1) enhanced hybridization kinetics; 2) tighter binding to complementary sequence than its unmodified counterpart; and/or, 3) resistance to nucleases. In another specific embodiment, the antisense drug may be a complementary oligonucleotide (DNA, RNA, or hybrid thereof), that has the ability to  
15 cleave its target sequence either by ribozyme, or ribozyme-like, activity, or by nuclease activity imparted on the antisense drug by physical attachment to anyone of a number of nucleases.

More specifically, the therapeutic method generally referred to herein could include the  
20 method for the treatment of various pathologies or other cellular dysfunctions and derangements by the administration of pharmaceutical compositions that may comprise effective inhibitors of eEF-2 kinase activity, or other equally effective drugs developed for instance by a drug screening assay prepared and used in accordance with a further aspect of the present invention.

25

Accordingly, it is a principal object of the present invention to provide a method and an associated assay system for screening potential inhibitors of eEF-2 kinase activity.

It is a further object of the present invention to provide antibodies to the phosphorylated  
30 eEF-2 kinase target. and methods for their preparation, including recombinant means.

It is a further object of the present invention to provide a method for detecting eEF-2 kinase activity in mammals in which invasive, spontaneous, or idiopathic pathological states are suspected to be present.

- 5 It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of eEF-2 kinase, so as to alter the adverse consequences of such presence or activity, or where beneficial, to enhance such activity.
- 10 It is a still further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of eEF-2 kinase, so as to treat or avert the adverse consequences of invasive, spontaneous or idiopathic pathological states.

- It is a still further object of the present invention to provide pharmaceutical
- 15 compositions for use in therapeutic methods which comprise or are based upon a sequence complementary to that of the eEF-2 kinase mRNA, which would form the basis for an antisense therapeutic that can reduce expression, and thus activity, of eEF-2 kinase.

- 20 It is yet another object of the invention to provide pharmaceutical compositions for use in therapeutic methods which comprise or are based upon peptide analogs of eEF-2 phosphorylation target amino acid sequences. It is anticipated that certain peptide analogs may act as efficacious competitive inhibitors of eEF-2 phosphorylation.

- 25 In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook"
- 30 Volumes I-III [J. E. Celis, ed. (1994)]; "Current Protocols in Immunology" Volumes I-

- III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984);  
"Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription  
And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I.  
Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press. (1986)]; B. Perbal,  
5 "A Practical Guide To Molecular Cloning" (1984).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

- 10 The terms "elongation factor-2 kinase", "eEF-2 kinase", "EF-2 kinase", "Cefk", and any  
variants not specifically listed, may be used herein interchangeably, and as used  
throughout the present application and claims refer to proteinaceous material including  
single or multiple proteins, and extends to those proteins having the amino acid sequence  
data described herein and presented in FIGURES 1 and 5 (SEQ ID NO: 2, 4, 6, 8, 10, 12,  
15 abd 14), and the profile of activities set forth herein and in the Claims. Accordingly,  
proteins displaying substantially equivalent or altered activity are likewise contemplated.  
These modifications may be deliberate, for example, such as modifications obtained  
through site-directed mutagenesis, or may be accidental, such as those obtained through  
mutations in hosts that are producers of the complex or its named subunits. Also, the  
20 terms elongation factor-2 kinase", "eEF-2 kinase", "EF-2 kinase", and "Cefk" are  
intended to include within their scope proteins specifically recited herein as well as all  
substantially homologous analogs and allelic variations.

- The amino acid residues described herein are preferred to be in the "L" isomeric form.  
25 However, residues in the "D" isomeric form can be substituted for any L-amino acid  
residue, as long as the desired fractional property of immunoglobulin-binding is retained  
by the polypeptide. NH<sub>2</sub> refers to the free amino group present at the amino terminus of  
a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus  
of a polypeptide. In keeping with standard polypeptide nomenclature, *J. Biol. Chem.*,

243:3552-59 (1969). abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

5	<u>SYMBOL</u>		<u>AMINO ACID</u>
	<u>1-Letter</u>	<u>3-Letter</u>	
	Y	Tyr	tyrosine
	G	Gly	glycine
	F	Phe	phenylalanine
10	M	Met	methionine
	A	Ala	alanine
	S	Ser	serine
	I	Ile	isoleucine
	L	Leu	leucine
15	T	Thr	threonine
	V	Val	valine
	P	Pro	proline
	K	Lys	lysine
	H	His	histidine
20	Q	Gln	glutamine
	E	Glu	glutamic acid
	W	Trp	tryptophan
	R	Arg	arginine
	D	Asp	aspartic acid
25	N	Asn	asparagine
	C	Cys	cysteine

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the



beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues. The above Table is presented to correlate the three-letter and one-letter notations which may appear alternately herein.

- 5 A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; *i.e.*, capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA  
10 segment may be attached so as to bring about the replication of the attached segment.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and  
15 does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of  
20 DNA (*i.e.*, the strand having a sequence homologous to the mRNA).

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

- 25 A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences,  
30 cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g.,

mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

25

A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide. N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal

sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

The term "oligonucleotide," as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use of the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the strand

to hybridize therewith and thereby form the template for the synthesis of the extension product.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to  
5 bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated  
10 (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome  
15 replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

20

Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard  
25 software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

It should be appreciated that also within the scope of the present invention are DNA sequences encoding eEF-2 kinase which code for a protein having the same amino acid sequence as SEQ ID NO: 2, 4, and 10, but which are degenerate to SEQ ID NO: 1, 3, and 9. By "degenerate to" is meant that a different three-letter codon is used to specify a particular amino acid. It is well known in the art that the following codons can be used interchangeably to code for each specific amino acid:

	Phenylalanine (Phe or F)	UUU or UUC
	Leucine (Leu or L)	UUA or UUG or CUU or CUC or CUA or CUG
10	Isoleucine (Ile or I)	AUU or AUC or AUA
	Methionine (Met or M)	AUG
	Valine (Val or V)	GUU or GUC or GUA or GUG
	Serine (Ser or S)	UCU or UCC or UCA or UCG or AGU or AGC
	Proline (Pro or P)	CCU or CCC or CCA or CCG
15	Threonine (Thr or T)	ACU or ACC or ACA or ACG
	Alanine (Ala or A)	GCU or GCG or GCA or GCG
	Tyrosine (Tyr or Y)	UAU or UAC
	Histidine (His or H)	CAU or CAC
	Glutamine (Gln or Q)	CAA or CAG
20	Asparagine (Asn or N)	AAU or AAC
	Lysine (Lys or K)	AAA or AAG
	Aspartic Acid (Asp or D)	GAU or GAC
	Glutamic Acid (Glu or E)	GAA or GAG
	Cysteine (Cys or C)	UGU or UGC
25	Arginine (Arg or R)	CGU or CGC or CGA or CGG or AGA or AGG
	Glycine (Gly or G)	GGU or GGC or GGA or GGG
	Tryptophan (Trp or W)	UGG
	Termination codon	UAA (ochre) or UAG (amber) or UGA (opal)

It should be understood that the codons specified above are for RNA sequences. The corresponding codons for DNA have a T substituted for U.

Mutations can be made in SEQ ID NO: 1, 3, and 9 such that a particular codon is changed  
5 to a codon which codes for a different amino acid. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (*i.e.*, by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping)  
10 or in a conservative manner (*i.e.*, by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The  
15 present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein.

The following is one example of various groupings of amino acids:

20

Amino acids with nonpolar R groups

Alanine

Valine

25 Leucine

Isoleucine

Proline

Phenylalanine

Tryptophan

30 Methionine

Amino acids with uncharged polar R groups

Glycine

Serine

5 Threonine

Cysteine

Tyrosine

Asparagine

Glutamine

10

Amino acids with charged polar R groups (negatively charged at pH 6.0)

Aspartic acid

Glutamic acid

15

Basic amino acids (positively charged at pH 6.0)

Lysine

Arginine

20 Histidine (at pH 6.0)

Another grouping may be those amino acids with phenyl groups:

Phenylalanine

25 Tryptophan

Tyrosine

Another grouping may be according to molecular weight (i.e., size of R groups):

30 Glycine

75



	Alanine	89
	Serine	105
	Proline	115
	Valine	117
5	Threonine	119
	Cysteine	121
	Leucine	131
	Isoleucine	131
	Asparagine	132
10	Aspartic acid	133
	Glutamine	146
	Lysine	146
	Glutamic acid	147
	Methionine	149
15	Histidine (at pH 6.0)	155
	Phenylalanine	165
	Arginine	174
	Tyrosine	181
	Tryptophan	204

20

Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- 25 - Gln for Asn such that a free NH<sub>2</sub> can be maintained.

Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly

30 "catalytic" site (*i.e.*, His can act as an acid or base and is the most common amino acid

in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces  $\beta$ -turns in the protein's structure.

Two amino acid sequences are "substantially homologous" when at least about 70% of  
5 the amino acid residues (preferably at least about 80%, and most preferably at least about 90 or 95%) are identical, or represent conservative substitutions.

A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature.  
10 Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than  
15 the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses polyclonal, monoclonal, and chimeric  
20 antibodies, the last mentioned described in further detail in U.S. Patent Nos. 4,816,397 and 4,816,567.

An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically  
25 binds antigen.

The phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contains the paratope, including those portions known in the art as Fab, Fab', F(ab')<sub>2</sub> and F(v), which portions are preferred for use in the therapeutic methods described herein.

5

Fab and F(ab')<sub>2</sub> portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous et al. Fab' antibody molecule portions are also well-known and are produced from F(ab')<sub>2</sub> portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

15 The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites,  
20 each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar  
25 untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably  
30 by at least 50 percent, most preferably by at least 90 percent, a clinically significant

change in the S phase activity of a target cellular mass, or other feature of pathology such as for example, elevated blood pressure, fever or white cell count as may attend its presence and activity.

- 5 A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the
- 10 expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

The term "standard hybridization conditions" refers to salt and temperature conditions

15 substantially equivalent to 5 x SSC and 65°C for both hybridization and wash. However, one skilled in the art will appreciate that such "standard hybridization conditions" are dependent on particular conditions including the concentration of sodium and magnesium in the buffer, nucleotide sequence length and concentration, percent mismatch, percent formamide, and the like. Also important in the determination of "standard hybridization

20 conditions" is whether the two sequences hybridizing are RNA-RNA, DNA-DNA or RNA-DNA. Such standard hybridization conditions are easily determined by one skilled in the art according to well known formulae, wherein hybridization is typically 10-20°C below the predicted or determined  $T_m$  with washes of higher stringency, if desired.

25

In one aspect, the present invention relates to the identification of a new superfamily of protein kinases centered around eEF-2 kinase. Accordingly, it includes the DNA sequences coding for these family members. In addition, the invention also contemplates that each member of this new protein kinase superfamily has its own cognate

phosphorylation target. As specified *supra*, two of these targets are eEF-2 and MHC, which are phosphorylated by eEF-2 kinase and MHCK A, respectively.

In a particular embodiment, the present invention relates to phosphorylation target  
5 analogs, which are short peptide sequences derived from phosphorylation targets of this new superfamily of protein kinases centered around eEF-2 kinase. Specifically, it is contemplated that these peptide analogs will be instrumental in the development of high throughput screening assays to identify inhibitors of members of this new superfamily.

10 As overexpression of eEF-2 kinase has been associated with a variety of cancers and other hyperproliferative pathologies (discussed *supra*), the invention also includes assay systems for the screening of potential drugs effective at inhibiting eEF-2 kinase activity. It is contemplated that any of the recited assays can be automated using technology that is standard to the skilled artisan.

15

As stated above, the present invention also relates to a recombinant DNA molecule or cloned gene, or a degenerate variant thereof, which encodes a eEF-2 kinase, or a fragment thereof, that possesses a molecular weight of about 100 kD and an amino acid sequence set forth in Figure 5 (SEQ ID NO: 2, 4, and 10); preferably a nucleic acid molecule, in  
20 particular a recombinant DNA molecule or cloned gene, encoding the 100 kD eEF-2 kinase has a nucleotide sequence or is complementary to a DNA sequence shown in Figure 5 (SEQ ID NO: 1, 3, and 9).

Therapeutic possibilities are raised by the knowledge of the eEF-2 kinase sequence and  
25 the existence of peptide analogs that can act as phosphorylation targets for the kinase. Accordingly, it is contemplated that sequences that are derived from the complement to the eEF-2 kinase mRNA sequence, and various modifications thereof, can act as potent antisense drugs that either inhibit expression in a competitive fashion, or, more effectively, by nuclease activity associated with the antisense drug that cleaves the eEF-2  
30 kinase mRNA sequence, thus rendering it irreversibly inactive. Alternative therapeutics

are also contemplated that concern the use of peptides and peptide analogs representing portions of phosphorylation target amino acid sequences. It is envisioned that such peptide-based drugs would inhibit eEF-2 kinase activity on its native target, thus bypassing the cascade of events that would lead to malignant transformation.

5

The antisense or peptide-based drugs may be prepared in pharmaceutical compositions, with a suitable carrier and at a strength effective for administration by various means to a patient experiencing an adverse medical condition associated with specific malignancies for the treatment thereof. A variety of administrative techniques may be utilized, among  
10 them parenteral techniques such as subcutaneous, intravenous and intraperitoneal injections, catheterizations and the like. Average quantities of the antisense or peptide-based drugs may vary and in particular should be based upon the recommendations and prescription of a qualified physician or veterinarian.

15 Also, antibodies including both polyclonal and monoclonal antibodies, and drugs that modulate the production or activity of eEF-2 kinase may possess certain diagnostic applications and may, for example, be utilized for the purpose of detecting and/or measuring levels of eEF-2 kinase. It is anticipated that further experimentation will reveal a prognostic correlation between eEF-2 kinase levels and the prediction and or  
20 progression of certain malignancies associated with carcinoma. For example, eEF-2 kinase may be used to produce both polyclonal and monoclonal antibodies to themselves in a variety of cellular media, by known techniques such as the hybridoma technique utilizing, for example, fused mouse spleen lymphocytes and myeloma cells. Likewise, small molecules that mimic or antagonize the activity of eEF-2 kinase of the invention  
25 may be discovered or synthesized, and may be used in diagnostic and/or therapeutic protocols.

The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal, antibody-producing cell lines can also be created by techniques other  
30 than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or

transfection with Epstein-Barr virus. See, e.g., M. Schreier et al., "Hybridoma Techniques" (1980); Hammerling et al., "Monoclonal Antibodies And T-cell Hybridomas" (1981); Kennett et al., "Monoclonal Antibodies" (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917;  
5 4,472,500; 4,491,632; 4,493,890.

Panels of monoclonal antibodies produced against eEF-2 kinase peptides can be screened for various properties; *i.e.*, isotype, epitope, affinity, etc. Of particular interest are monoclonal antibodies that neutralize the activity of eEF-2 kinase. Such monoclonals  
10 can be readily identified in eEF-2 kinase activity assays. High affinity antibodies are also useful when immunoaffinity purification of native or recombinant eEF-2 kinase is desired.

Preferably, the anti-eEF-2 kinase antibody used in the diagnostic methods of this  
15 invention is an affinity purified polyclonal antibody. More preferably, the antibody is a monoclonal antibody (mAb). In addition, it is preferable for the anti-eEF-2 kinase antibody molecules used herein be in the form of Fab, Fab', F(ab')<sub>2</sub> or F(v) portions of whole antibody molecules.

20 As suggested earlier, the diagnostic method of the present invention comprises examining a cellular sample or medium by means of an assay including an effective amount of an antagonist to eEF-2 kinase, such as an anti-eEF-2 kinase antibody, preferably an affinity-purified polyclonal antibody, and more preferably a mAb. In addition, it is preferable for the anti-eEF-2 kinase antibody molecules used herein be in the form of Fab, Fab', F(ab')<sub>2</sub>  
25 or F(v) portions or whole antibody molecules. As previously discussed, patients capable of benefiting from this method include those suffering from cancer, a pre-cancerous lesion, a viral infection or other like pathological derangement. Methods for isolating the eEF-2 kinase and inducing anti-eEF-2 kinase antibodies and for determining and optimizing the ability of anti-eEF-2 kinase antibodies to assist in the examination of the  
30 target cells are all well-known in the art.



Methods for producing polyclonal anti-polypeptide antibodies are well-known in the art. See U.S. Patent No. 4,493,795 to Nestor et al. A monoclonal antibody, typically containing Fab and/or F(ab')<sub>2</sub> portions of useful antibody molecules, can be prepared using the hybridoma technology described in *Antibodies - A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory, New York (1988), which is incorporated  
5 herein by reference.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 6000. Fused hybrids are selected by their sensitivity to HAT. Hybridomas producing a  
10 monoclonal antibody useful in practicing this invention are identified by their ability to immunoreact with the present eEF-2 kinase and their ability to inhibit specified eEF-2 kinase activity in target cells.

A monoclonal antibody useful in practicing the present invention can be produced by  
15 initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate antigen specificity. The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well-known  
20 techniques.

Media useful for the preparation of these compositions are both well-known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential medium (DMEM;  
25 Dulbecco et al., *Virology* 8:396 (1959)) supplemented with 4.5 gm/l glucose, 20mM glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

Methods for producing monoclonal anti-eEF-2 kinase antibodies are also well-known in the art. See Niman et al., *Proc. Natl. Acad. Sci. USA*, 80:4949-4953 (1983). Typically,  
30 the present eEF-2 kinase or a peptide analog is used either alone or conjugated to an

immunogenic carrier, as the immunogen in the before described procedure for producing anti-eEF-2 kinase monoclonal antibodies. The hybridomas are screened for the ability to produce an antibody that immunoreacts with the eEF-2 kinase peptide analog and the present eEF-2 kinase.

5

The present invention further contemplates therapeutic compositions useful in practicing the therapeutic methods of this invention. A subject therapeutic composition includes, in admixture, a pharmaceutically acceptable excipient (carrier) and one or more of an anti-eEF-2 kinase antibody, peptide analog capable of competing for phosphorylation of eEF-2 by eEF-2 kinase, antisense drug against eEF-2 kinase mRNA, or any other  
10 compound that is found to inhibit eEF-2 kinase activity. In a preferred embodiment, the composition comprises an antigen capable of modulating the activity of eEF-2 kinase within a target cell.

15 The preparation of therapeutic compositions which contain polypeptides, analogs or active fragments as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic  
20 ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active  
25 ingredient.

A polypeptide, analog or active fragment can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the  
30 polypeptide or antibody molecule) and which are formed with inorganic acids such as,

for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 5 2-ethylamino ethanol, histidine, procaine, and the like.

The therapeutic polypeptide-, analog- or active fragment-containing compositions are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present 10 invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; *i.e.*, carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, 15 and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition or neutralization of eEF-2 kinase activity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may 20 range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other 25 administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

### Formulations

#### 30 Intravenous Formulation I

	<u>Ingredient</u>	<u>mg/ml</u>
	cefotaxime	250.0
	antibody, peptide, antisense drug, or other compound	10.0
	dextrose USP	45.0
5	sodium bisulfite USP	3.2
	edetate disodium USP	0.1
	water for injection q.s.a.d.	1.0 ml
<u>Intravenous Formulation II</u>		
10	<u>Ingredient</u>	<u>mg/ml</u>
	ampicillin	250.0
	antibody, peptide, antisense drug, or other compound	10.0
	sodium bisulfite USP	3.2
	disodium edetate USP	0.1
15	water for injection q.s.a.d.	1.0 ml
<u>Intravenous Formulation III</u>		
	<u>Ingredient</u>	<u>mg/ml</u>
	gentamicin (charged as sulfate)	40.0
20	antibody, peptide, antisense drug, or other compound	10.0
	sodium bisulfite USP	3.2
	disodium edetate USP	0.1
	water for injection q.s.a.d.	1.0 ml
25	<u>Intravenous Formulation IV</u>	
	<u>Ingredient</u>	<u>mg/ml</u>
	antibody, peptide, antisense drug, or other compound	10.0
	dextrose USP	45.0
	sodium bisulfite USP	3.2
30	edetate disodium USP	0.1

water for injection q.s.a.d.

1.0 ml

As used herein, "pg" means picogram. "ng" means nanogram, "ug" or "μg" mean microgram, "mg" means milligram, "ul" or "μl" mean microliter, "ml" means milliliter,  
5 "l" means liter.

Another feature of this invention is the expression of the DNA sequences disclosed herein. As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and  
10 employing that expression vector to transform an appropriate unicellular host.

Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of an initiation codon. ATG, in the correct reading frame upstream of the DNA sequence.

15

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli*  
20 plasmids col El, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAs, e.g., the numerous derivatives of phage λ, e.g., NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and  
25 phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

Any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence operatively linked to it -- may be used in these vectors to  
30 express the DNA sequences of this invention. Such useful expression control sequences

include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the *LTR* system, the major operator and promoter regions of phage  $\lambda$ , the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the  
5 promoters of acid phosphatase (e.g., Pho5), the promoters of the yeast  $\alpha$ -mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

A wide variety of unicellular host cells are also useful in expressing the DNA sequences  
10 of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, R1.1, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture.

15

It will be understood that not all vectors, expression control sequences and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts  
20 without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.

25

In selecting an expression control sequence, a variety of factors will normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular  
30 hosts will be selected by consideration of, e.g., their compatibility with the chosen vector,



their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products.

5

Considering these and other factors, a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the DNA sequences of this invention on fermentation or in large scale animal culture.

- 10 The present invention extends to the preparation of antisense oligonucleotides and ribozymes that may be used to interfere with the expression of the eEF-2 kinase gene at the translational level. This approach utilizes antisense nucleic acid and ribozymes to block translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or cleaving it with a ribozyme.

15

- Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule. (See Weintraub, 1990; Marcus-Sekura, 1988.) In the cell, they hybridize to that mRNA, forming a double stranded molecule. The cell does not translate an mRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression of mRNA into protein. Oligomers of about fifteen nucleotides and molecules that hybridize to the AUG initiation codon will be particularly efficient, since they are easy to synthesize and are likely to pose fewer problems than larger molecules when introducing them into eEF-2 kinase-producing cells. Antisense methods have been used to inhibit the expression of many genes *in vitro*
- 20
- 25 (Marcus-Sekura, 1988; Hambor et al., 1988).

- Ribozymes are RNA molecules possessing the ability to specifically cleave other single stranded RNA molecules in a manner somewhat analogous to DNA restriction endonucleases. Ribozymes were discovered from the observation that certain mRNAs
- 30 have the ability to excise their own introns. By modifying the nucleotide sequence of

these RNAs, researchers have been able to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988.). Because they are sequence-specific, only mRNAs with particular sequences are inactivated.

5 Investigators have identified two types of ribozymes, *Tetrahymena*-type and "hammerhead"-type. (Hasselhoff and Gerlach, 1988) *Tetrahymena*-type ribozymes recognize four-base sequences, while "hammerhead"-type recognize eleven- to eighteen-base sequences. The longer the recognition sequence, the more likely it is to occur exclusively in the target mRNA species. Therefore, hammerhead-type ribozymes  
10 are preferable to *Tetrahymena*-type ribozymes for inactivating a specific mRNA species, and eighteen base recognition sequences are preferable to shorter recognition sequences.

The DNA sequences described herein may thus be used to prepare antisense molecules against, and ribozymes that cleave mRNAs for eEF-2 kinase .

15

The present invention also relates to a variety of diagnostic applications, including methods for detecting and quantifying the levels of eEF-2 kinase. As mentioned earlier, eEF-2 kinase can be used to produce antibodies to itself by a variety of known techniques, and such antibodies could then be isolated and utilized as in tests for the  
20 presence and levels of eEF-2 kinase activity in suspect target cells.

As described in detail above, antibody(ies) to eEF-2 kinase can be produced and isolated by standard methods including the well known hybridoma techniques. For convenience, the antibody(ies) to eEF-2 kinase will be referred to herein as Ab<sub>1</sub> and antibody(ies)  
25 raised in another species as Ab<sub>2</sub>.

The presence and levels of eEF-2 kinase in cells can be ascertained by the usual immunological procedures applicable to such determinations. A number of useful procedures are known. Three such procedures which are especially useful, utilize either  
30 eEF-2 kinase labeled with a detectable label, antibody Ab<sub>1</sub> labeled with a detectable label,

or antibody  $Ab_2$  labeled with a detectable label. The procedures may be summarized by the following equations wherein the asterisk indicates that the particle is labeled, and "~" stands for eEF-2 kinase:

- A.  $\sim^* + Ab_1 = \sim^* Ab_1$   
 5 B.  $\sim + Ab^* = \sim Ab_1^*$   
 C.  $\sim + Ab_1 + Ab_2^* = \sim Ab_1 Ab_2^*$

The procedures and their application are all familiar to those skilled in the art and accordingly may be utilized within the scope of the present invention. The "competitive"  
 10 procedure, Procedure A, is described in U.S. Patent Nos. 3,654,090 and 3,850,752. Procedure C, the "sandwich" procedure, is described in U.S. Patent Nos. RE 31,006 and 4,016,043. Still other procedures are known such as the "double antibody," or "DASP" procedure.

- 15 In each instance, eEF-2 kinase forms complexes with one or more antibody(ies) or binding partners and one member of the complex is labeled with a detectable label. The fact that a complex has formed and, if desired, the amount thereof, can be determined by known methods applicable to the detection of labels.
- 20 It will be seen from the above, that a characteristic property of  $Ab_2$  is that it will react with  $Ab_1$ . This is because  $Ab_1$  raised in one mammalian species has been used in another species as an antigen to raise the antibody  $Ab_2$ . For example,  $Ab_2$  may be raised in goats using rabbit antibodies as antigens.  $Ab_2$  therefore would be anti-rabbit antibody raised in goats. For purposes of this description and claims,  $Ab_1$  will be referred to as a primary  
 25 or anti-eEF-2 kinase antibody, and  $Ab_2$  will be referred to as a secondary or anti- $Ab_1$  antibody.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.

A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

5

eEF-2 kinase can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^{36}\text{Cl}$ ,  $^{51}\text{Cr}$ ,  $^{57}\text{Co}$ ,  $^{58}\text{Co}$ ,  $^{59}\text{Fe}$ ,  $^{90}\text{Y}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ , and  $^{186}\text{Re}$ .

10

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase,  $\beta$ -glucuronidase,  $\beta$ -D-glucosidase,  $\beta$ -D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

20

A particular assay system developed and utilized in accordance with the present invention, is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the labeled and unlabeled material after which binding studies are conducted to determine the extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

25

Accordingly, a purified quantity of the eEF-2 kinase may be radiolabeled and combined, for example, with antibodies or other inhibitors thereto, after which binding studies would be carried out. Solutions would then be prepared that contain various quantities

30

of labeled and unlabeled uncombined eEF-2 kinase, and cell samples would then be inoculated and thereafter incubated. The resulting cell monolayers are then washed, solubilized and then counted in a gamma counter for a length of time sufficient to yield a standard error of <5%. These data are then subjected to Scatchard analysis after which  
5 observations and conclusions regarding material activity can be drawn. While the foregoing is exemplary, it illustrates the manner in which a receptor assay may be performed and utilized, in the instance where the cellular binding ability of the assayed material may serve as a distinguishing characteristic.

10 In accordance with the above, an assay system for screening potential drugs effective to modulate the activity of eEF-2 kinase may be prepared. The eEF-2 kinase may be introduced into a test system, and the prospective drug may also be introduced into the resulting cell culture, and the culture thereafter examined to observe any changes in the eEF-2 kinase activity of the cells, due either to the addition of the prospective drug alone,  
15 or due to the effect of added quantities of the known eEF-2 kinase. Alternatively, these assays can be carried out in a purely *in vitro* fashion as discussed below.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting  
20 the broad scope of the invention.

## EXPERIMENTAL DETAILS SECTION

### EXAMPLE 1

25 **Peptide Sequencing.** eEF-2 kinase from rabbit reticulocyte lysate was purified as described (Hait et al., (1996) *FEBS Lett.* 397:55-60). Peptides were generated from the nitrocellulose-bound 103-kDa eEF-2 kinase protein by *in situ* tryptic digestion (Erdjument-Bromage et al., (1994) *Protein Sci.* 3:2435-2446) and fractionated by reverse-phase HPLC (Elicone et al., (1994) *J. Chromatogr.* 676:121-137) using a 1.0 mm Reliasil  
30 C18 column. Selected peak fraction were then analyzed by a combination of automated

Edman sequencing and matrix-assisted laser-desorption time-of-flight mass spectrometry (Erdjument-Bromage et al., (1994)). The peptide sequences provided an essential lead into the cloning of eEF-2 kinase from human, mouse, rat, and *Caenorhabditis elegans*.

5

## EXAMPLE 2

**Molecular Cloning of cDNAs Encoding *C. elegans*, Mouse, Rat, and Human eEF-2 Kinases.** To clone the cDNA for *C. elegans* eEF-2 kinase, oligonucleotide primers were designed based on the amino and carboxy termini of the predicted gene product from F42A10.4. Reverse transcriptase-PCR (RT-PCR) was performed using these primers and  
10 total RNA from *C. elegans* ( a gift from Monica Driscoll, Rutgers University). A single PCR product of ~2.3 kb was obtained and gel-purified using a gel extraction kit (Qiagen, Chatsworth, CA). The fragment was ligated into vector pCR2.1 using the TA cloning kit (Invitrogen, Sorrento Valley, CA), and then transformed into *Escherichia coli*. Plasmid DNA was purified, and restriction analysis used to verify the orientation of the coding  
15 sequence with respect to the T7 promoter. Two clones (*Cefk-1* and *Cefk-2*, *C. elegans* eEF-2 kinase isoforms 1 and 2) were chosen and sequenced using a Li-Cor (Lincoln, NE) Long Read IR model 400L Automated DNA Sequencer. Analysis revealed that the two clones were identical except for a deletion of 24 bp in *Cefk-2* which corresponds to exon 4 and probably represents an alternatively spliced form.

20

To clone the mouse eEF-2 kinase, degenerate primers were designed based on the amino acid sequence of two peptides from rabbit eEF-2 kinase (LTPQAFSHFTFER (SEQ ID NO: 21) and LANXYYEKAE (SEQ ID NO: 22)): primer A, CA(G/A)GC(C/G/T/A)TT(C/T)(T/A)(C/G)(T/CCA(C/T)TT(C/T)AC(C/G/T/A)TT(C/  
25 T)GA(G/A(C/A)G (SEQ ID NO: 23); and primer B, TC(C/G/T/A)GC(C/T)TT(C/T)TC(G/A)TA(G/A)TA(C/T)TT(G/A)TT(C/G/A/T)GC (SEQ ID NO: 24). RT-PCR was performed using primers A and B and poly(A)<sup>+</sup> RNA from mouse spleen (CLONTECH). A single PCR product (~1.6 kb) was cloned into pCR2.1 (Invitrogen) and sequenced. Using sequence information from these mouse eEF-  
30 2 kinase cDNA fragments, new primers were designed for 5' rapid amplification of



cDNA ends (RACE) and 3' RACE to obtain full-length mouse eEF-2 kinase cDNA. 5' RACE and 3' RACE were performed using Marathon-Ready mouse spleen cDNA (CLONTECH). This was carried out according to the manufacturer's instructions using the primers AP1 and C (TACAATCAGCTGATGACCAGAACGCTC) (SEQ ID NO: 25) 5' antisense, or D (GGATTTGGACTGGACAAGAACCCCC) (SEQ ID NO: 19) 3' sense.

To clone rat eEF-2 kinases, PCR was performed on a rat PC12 cDNA library cloned in  $\lambda$ GT10 (CLONTECH) using primer B and vector primers. A 700-bp fragment was specifically amplified. The fragment was cloned into pCR2.1 (Invitrogen) and sequenced. This 700-bp fragments was radiolabeled and used to probe the same PC12 cDNA library (600,000 plaques). Fourteen positives were obtained in the initial screening. Five plaques were chosen for further analysis and sequencing based on insert sizes that ranged from 1.4 to 2.0 kb.

15

Recently, eEF-2 kinase from rabbit reticulocyte lysate was purified to near homogeneity (Hait et al., (1996)). This enabled determination of its partial amino acid sequence (see EXAMPLE 1). Two peptide sequences (LTPQAFSHFTFER and LANXYYEKAE) were compared with entries in a nonredundant database using the National Center for Biotechnology Information BLAST program (Altschul et al., (1990) *J. Mol. Biol.* 215:403-410). Matches were found with a *C. elegans* hypothetical protein (F42A10.4; GenBank accession number U10414). This sequence was obtained from the *C. elegans* genome sequencing project and is located on chromosome III (Wilson et al., (1994) *Nature* 368:32-38). The 100% identity between the sequenced peptides and the *C. elegans* protein, as well as the fact that the predicted molecular weight of the *C. elegans* protein is similar to that of eEF-2 kinase, suggested that this gene encoded eEF-2 kinase. The full-length cDNA by RT-PCR using *C. elegans* total RNA was cloned. Several clones were isolated and sequenced. *Cefk-1* has six of the predicted exons and encodes 768 amino acids. *Cefk-2* represents an alternatively spliced form that has five exons; it is missing amino acids 625-632 that correspond to exon four.

30



As is demonstrated in EXAMPLE 3, *Cefk-1* and *Cefk-2* have eEF-2 kinase activity when expressed in cell-free system using a wheat germ extract coupled transcription/translation system.

- 5 To determine the amino acid sequence of mammalian eEF-2 kinase, the cDNA of mouse eEF-2 kinase was cloned and sequenced. Since the sequenced peptides from rabbit eEF-2 were 100% identical to *C. elegans* eEF-2 kinase, then the two peptides should also match the sequence of mouse eEF-2 kinase. Degenerate primers were designed based on the amino acid sequence of the peptides and were used to perform
- 10 RT-PCR on mouse spleen poly(A)<sup>+</sup> mRNA. A single PCR product of ~1.6 kb was obtained and sequenced. To obtain the full-length cDNA, 5' RACE and 3' RACE were performed using mouse spleen cDNA. The full-length cDNA, which encodes 724 amino acids, was expressed in a cell-free coupled transcription/translation system. A single translation product with an apparent molecular weight of 100 kDa was obtained
- 15 (Figure 2).

- cDNA for rat eEF-2 kinase using a fragment of mouse eEF-2 kinase cDNA to probe a PC12 cDNA library was cloned and sequenced. However, after this work was completed, a paper describing the cloning of eEF-2 from rat skeletal muscle was
- 20 published (Redpath et al., (1996) *J. Biol. Chem.* 271:17547-17554) and the reported sequence appears to be identical to the eEF-2 kinase sequence from PC12 cells. Like the mouse eEF-2 kinase, the rat eEF-2 kinase cDNA encodes a 724-amino acid protein.

- The human eEF-2 kinase cDNA was cloned and sequenced. RT-PCR was performed
- 25 on poly(A)<sup>+</sup> mRNA from the human glioma cell line T98G using 20' mer primers corresponding to the 5' and 3' ends of the mouse eEF-2 kinase coding region. The human eEF-2 kinase cDNA encodes a 725 amino acid protein.

### EXAMPLE 3

**Expression of eEF-2 Kinase From *C. elegans*, Mouse, Rat, and Human in a Cell-Free System.** Plasmid DNA from clones *Cefk-1*, *Cefk-2*, as well as mouse and human eEF-2 kinase cDNA were used in the TNT wheat germ extract coupled  
5 transcription/translation system (Promega). [<sup>35</sup>S]Methionine-labeled products were then analyzed by SDS/PAGE. The reaction mixture (50 µl total volume) contained 1 µg of plasmid DNA and 26 µCi of [<sup>35</sup>S]methionine (specific activity = 1175.0 Ci/mmol; 1 Ci = 37 GBq). Other components were added to the reaction mixture according to the manufacturer's protocol. The reaction mixture was incubated for 1.5 h at 30°C and  
10 terminated by incubation on ice. A 10 µl aliquot of the reaction mixture was mixed with 2 µl of 5X Laemmli buffer and boiled for 5 min. Samples were analyzed by SDS/PAGE on 8% gels and autoradiography.

The remainder of the transcription/translation reaction was diluted 4-fold with buffer A  
15 (20 mM Tris-HCl, pH 7.4/1 mM MgCl<sub>2</sub>/10% glycerol/7 mM 2-mercaptoethanol) and applied to a HR5/5 Mono Q column (Pharmacia) equilibrated with buffer A. The column was developed with 20 column volumes of a 50-600 mM KCl linear gradient to buffer A.

20 To assay for eEF-2 kinase activity, 5 µl from each fraction was added to a reaction mixture (40 µl) containing 50 mM Hepes-KOH (ph. 7.4) 10 mM magnesium acetate, 0.1 mM CaCl<sub>2</sub>, 5 mM dithiothreitol, 50 µM ATP, 2 µCi [ $\gamma$ -<sup>32</sup>P]ATP, 0.6 µg calmodulin, and 0.5 µg rabbit reticulocyte eEF-2. Reactions were incubated at 30°C for 2 min and were  
25 terminated by adding 20 µl of 3X Laemmli sample buffer. Samples were boiled for 5 min and proteins separated by SDS/PAGE on 8% gels. Phosphoproteins were analyzed by autoradiography.

To determine whether *Cefk-1* and *Cefk-2* have eEF-2 kinase activity, cell-free coupled transcription/translation system were expressed. Translation of *Cefk-1* and *Cefk-2*  
30 produced products with an apparent molecular weight of 100 kDa (Figure 2), which is

slightly larger than the computer-predicted molecular weight of the protein but is identical to the molecular weight of a rabbit reticulocyte eEF-2 kinase as determined by SDS/PAGE. The translation products of the mixture of *Cefk-1* and *Cefk-2* are able to phosphorylate eEF-2 (Figure 3) and elute from a Mono Q column at the same position as endogenous *C. elegans* eEF-2 kinase (Figure 3A). The eEF-2 phosphorylation activity of the recombinant protein is  $\text{Ca}^{2+}$ /calmodulin-dependant (Figure 3C). The differences in the catalytic properties *Cefk-1* and *Cefk-2* isoforms are under current investigation.

Mouse and human eEF-2 kinase cDNAs were expressed in a coupled transcription/translation system and a product of ~100 kDa was obtained (Figure 2). As shown in Figure 3, the recombinant human eEF-2 kinase activity was strictly  $\text{Ca}^{2+}$ /calmodulin-dependant. The kinase activity was completely inhibited by the calmodulin antagonists trifluoperazine and *N*-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide. Human eEF-2 kinase in bacteria as a glutathione S-transferase fusion protein was expressed and demonstrated that the ability of the recombinant enzyme to phosphorylate eEF-2 and to undergo autophosphorylation are strictly calmodulin-dependent.

20

#### EXAMPLE 4

**Analysis of Mouse eEF-2 Kinase mRNA Expression in Various Tissues.** eEF-2 kinase and eEF-2 hybridizations were performed using a 1.6 kb *EcoRI* mouse cDNA fragment and a 2.6 kb *EcoRI* human cDNA fragment, respectively. cDNAs were labeled with [ $^{32}\text{P}$ ]dCTP using the random-primed DNA labeling method (Feinberg and Vogelstein (1983) *Anal. Biochem.* 132:6-13). A multiple tissue Northern blot (CLONETECH) was prehybridized at 42°C for 16 h in a 50% formamide solution containing 10X Denhardt's, 5X SSPE, 2% SDS, and 100 µg/ml salmon sperm DNA. Hybridizations were completed in the same solution containing the  $^{32}\text{P}$ -labeled probe ( $1 \times 10^6$  cpm/ml; specific activity,  $\sim 1 \times 10^8$  dpm/µg DNA) and 10% dextran sulfate at 42°C for 16 h. Blots were washed twice at room temperature (15 min) in 2X SSPE, 0.05%

SDS, and once at 50°C (15 min) in 0.5X SSPE, 0.5% SDS. RNA/cDNA hybrids were visualized by autoradiography.

Northern blot analysis shows that eEF-2 kinase is ubiquitously expressed in mouse tissues and is particularly abundant in skeletal muscle and heart (Figure 4). The abundance of eEF-2 kinase mRNA in muscle tissues may indicate that phosphorylation of eEF-2 is particularly important in muscle, or that there are additional substrates of eEF-2 kinase which are muscle-specific.

10

#### EXAMPLE 5

**Lack of Homology of eEF-2 Kinase to Members of Eukaryotic Protein Kinase Superfamily.** The alignment of the amino acid sequences of *C. elegans* and mammalian eEF-2 kinases is shown in Figure 5. Rat and mouse eEF-2 kinase are very similar being 97% identical and differing by only 23 amino acids. Human eEF-2 kinase is 90% identical to mouse and rat eEF-2 kinase. In contrast, *C. elegans* eEF-2 kinase is found to be only 40% identical to mammalian eEF-2 kinase.

According to the current classification, eEF-2 kinase belongs to the family of closely related calmodulin-dependent protein kinases. Surprisingly, upon analyzing eEF-2 kinase sequences, any homology to the other calmodulin-dependent kinases or to any other members of the protein kinase super-family was not found. The only motif which it shares with all other protein kinases is the GXGXXG motif (279-284 in *C. elegans* eEF-2 kinases; 295-300 in mouse eEF-2 kinase) which forms a glycine-rich loop and is part of the ATP-binding site. Comparison of mammalian and *C. elegans* eEF-2 kinase revealed only one extended region of homology that spans ~200 amino acids upstream of the GXGXXG motif. The high degree of similarity and the proximity to the nucleotide-binding site suggests that these 200 amino acids represent the catalytic domain. This region has a high degree of similarity and a portion of this region (amino acids 251-300 in mouse eEF-2 kinase) displays 75% identity to the catalytic domain of

MHCKA (see below), which also suggests that this is the catalytic domain. In the recently published rat eEF-2 kinase sequence [Redpath *et al.*, *J. Biol. Chem.* **271**: 17547-17554 (1996)], the catalytic domain was predicted to reside between amino acids 288 and 554 based on the homology with the catalytic domain of cAMP-dependant protein kinase (PKA). The results demonstrate that their prediction cannot be correct for several reasons. First, the homology of this region with PKA is not statistically significant. Second, this region is the least conserved between mammalian and *C. elegans* eEF-2 kinase. Finally, according to secondary structure predictions [made by Alexei V. Finkelstein, Institute of Protein Research, Russia using the ALB-GLOBULE program [Ptitsyn and Finkelstein, *Biopolymers* **22**:15-25 (1983)]], this region most likely has a distorted structure and contains almost no  $\alpha$ -helices or  $\beta$ -strands, which are characteristic of a catalytic domain.

Because eEF-2 kinase is  $Ca^{2+}$ /calmodulin-dependant, it should contain a calmodulin-binding domain, which is usually represented by an amphipathic  $\alpha$ -helix. There are several regions that could possibly assume an amphipathic  $\alpha$ -helical conformation. Further biochemical analysis is required to determine which of these is the calmodulin-binding domain.

In the C-terminal region, there is a short stretch of 22 amino acids which is 86% identical between mammalian and *C. elegans* eEF-2 kinase and is preceded by a longer region of weak homology. One of the possibilities is that it is involved in oligomerization of the kinase. It was thought previously that eEF-2 kinase was an elongated monomer because it migrated during gel filtration as an ~150-kDa protein and migrated on SDS gels as a 105-kDa polypeptide [Ryazanov and Spirin, *Translational Regulation of Gene Expression*, Plenum, NY, Vol 2, pp 433-455 (1993); Abdelnajid *et al.*, *Int. J. Dev. Biol.*, **37**:279-290 (1993)]. However, the molecular weight of a monomer of mammalian eEF-2 kinase based on the predicted sequence is just 82 kDa. Thus, it is possible that eEF-2 kinase is not a monomer but a responsible for dimerization. Interestingly, according to computer prediction using the COIL

program, this conserved region can form a coiled-coil. Formation of coiled-coil is often responsible for dimerization [Lupas, *Trends Biochem. Sci.*, 21:375-382 (1996)].

5

#### EXAMPLE 6

**Striking Homology Between eEF-2 Kinase and MHCK A from *Dictyostelium*.** It was found that eEF-2 kinases is homologous to the central portion of the recently described MHCKA from *Dictyostelium* [Futey *et al.*, *J. Biol. Chem.* 270:523-529 (1995) see Figure 5]. The kinase was biochemically identified as a 130-kDa protein and has a demonstrated role in myosin assembly, both *in vitro* and *in vivo* [Futey *et al.*, 1995, *supra*]. As with eEF-2 kinase, MHCKA displays no region with detectable similarity to the conserved catalytic domains found in known eukaryotic protein kinases. Primary structure analysis of MHCKA revealed an amino-terminal domain with a probable coiled-coil structure, a central nonrepetitive domain, and a C-terminal domain consisting of seven WD repeats [Futey *et al.*, 1995, *supra*]. A fragment of the central nonrepetitive domain of MHCKA containing amino acids 552-841 was recently shown to represent the catalytic domain [Cote *et al.*, *J. Biol. Chem.* 272:6846-6849 (1997)].

20 Because the catalytic domain of MHCKA and eEF-2 kinase have a high degree of similarity, the substrate specificity of these two kinases was assayed. Figure 6 shows that MHCK A cannot phosphorylate eEF-2, and likewise, rabbit eEF-2 kinase cannot use myosin heavy chains as a substrate. This demonstrated that each of these kinases is specific for their respective substrates.

25

#### EXAMPLE 7

**eEF-2 Kinase and MHCK A Define a New Class of Protein Kinases.** Members of the eukaryotic protein kinase superfamily are characterized by a conserved catalytic domain containing approximately 260 amino acids and is divided into twelve

30



subdomains [Hanks and Hunter, *FASEB J.*, 9:576-596 (1996); Hardie and Hanks, *The Protein Kinase Facts Book*, Academic, London (1995), Taylor *et al.*, *Annu. Rev. Cell Biol.* 8:429-462 (1992) Johnson *et al.*, *Cell.* 85: 149-158 (1996)]. The three-dimensional structure of several protein kinases revealed that the catalytic domain  
5 consists of two lobes. The smaller N-terminal lobe, which has a twisted  $\beta$ -sheet structure, represents the ATP-binding domain. The larger C-terminal lobe, which is predominantly  $\alpha$ -helical is involved in substrate binding. At the primary structure level, the only motif similar between eEF-2 kinase, MHCK A, and other protein kinases is the GXGXXG motif which forms the loop interacting directly with the  
10 phosphates of ATP [Hanks and Hunter, 1996, *supra*; Hardie and Hanks 1995, *supra*; Taylor *et al.*, *supra*]. In eukaryotic protein kinases, this motif is located at the very N terminus of the ATP-binding lobe of the catalytic domain. In contrast, in a eEF-2 kinase and MHCK A, this motif is close to the C terminus of the catalytic domain (see Figure 7). However, the overall topology of the ATP-binding subdomain of eEF-2  
15 kinase and MHCK A can be similar to other protein kinases because the region upstream of the GXGXXG motif is strongly predicted to contain four or five  $\beta$ -strands and thus can form a twisted  $\beta$ -sheet.

However, the mechanism of ATP-binding to eEF-2 kinase is probably quite different  
20 in comparison to other conventional members of the eukaryotic protein kinase superfamily. In protein kinases, there is a conserved lysine residue, corresponding to Lys-72 in cAMP-dependant protein kinases which binds to the  $\beta$ - and  $\gamma$ -phosphates of ATP and is located at about 20 amino acids downstream of the GXGXXG motif. Analysis of eEF-2 kinase and MHCK A sequences revealed that there are no conserved  
25 lysine residues in the vicinity of the GXGXXG motif. There is another atypical protein kinase, BCR-ABLE, which does not contain this conserved lysine and it is proposed that it interacts with ATP *via* two cysteine residues [Maro and Witte, *Cell*, 67:459-468 (1991)]. Interestingly, eEF-2 kinase and MHCK-A contain two conserved cysteine residues (Cys-313 and Cys-317 in mouse eEF-2 kinase) which are located near the  
30 GXGXXG motif and therefore might be involved in ATP binding. Thus the



mechanism of ATP-binding of eEF-2 kinase and MHCK A is different from other members of the protein kinase superfamily, but may be similar to that of the BCR-ABLE protein kinase.

- 5 The overall catalytic mechanism of eEF-2 kinase and MHCKA is probably also very different from other eukaryotic protein kinases. All members of the eukaryotic protein kinase superfamily contain a DXXXN motif in the catalytic loop and a DFG motif in the activation segment [Hanks and Hunter, 1996; *supra*, Hardie and Hanks 1995, *supra*; Taylor *et al.*, *supra*; Johnson *et al.*, 1996, *supra*]. These two motifs, which are  
10 directly involved in the catalysis of the protein phosphorylation reaction, are absent from the eEF-2 kinase and MHCK A catalytic domain.

- It is not known whether there are other protein kinases which are structurally similar to eEF-2 kinase and MHCK A. An extensive search of the entire nonrestricted  
15 database of the National Center for Biotechnology Information using the BLAST program did not reveal any protein with a significant homology to the catalytic domain of eEF-2 kinase and MHCKA. A search of the Expressed Sequence Tag (EST) database revealed several ESTs from *C. elegans*, mouse and human which are essentially identical to portions of eEF-2 kinase cDNA sequences reported here.  
20 Interestingly, a search of the recently completed genome database of *Saccharomyces cerevisiae* did not reveal any protein with homology to eEF-2 kinase despite the fact that eEF-2 phosphorylation was reported in yeast (41).

- Conclusion.** Since the catalytic domains of eEF-2 kinase and MHCK A do not share  
25 homology with other known protein kinases, these two protein kinases establish the presence of a novel and widespread superfamily of eukaryotic protein kinases. Although the existence of several unusual protein kinases have been reported, to the knowledge, it was demonstrated for the first time the existence of a biochemically well-characterized and ubiquitous protein kinase that is structurally unrelated to other  
30 serine/threonine/tyrosine kinases. Contrary to the widely accepted belief that all

eukaryotic protein kinases evolved from a single ancestor, the results suggest that eukaryotic protein kinases appeared at least twice during the course of evolution. This also suggests that, in addition to the relatively well-characterized catalytic mechanism employed by members of eukaryotic serine/threonine/tyrosine protein kinase superfamily, there exists another mechanism of protein kinase superfamily, there exists another mechanism of protein phosphorylation. Further studies will reveal the molecular details of this mechanism and whether there are other protein kinases that phosphorylate their substrates using this mechanism.

10

### EXAMPLE 8

**Preparation of recombinant eEF-2 kinase fusion proteins with GST, 6xHis, and thioredoxin.** Human eEF-2 kinase cDNA was cloned into three different expression vectors: pGEX-2T (Pharmacia Biotech, Piscataway, NJ); pRSET A (Invitrogen, Sorrento Valley, CA); and, pThioHisB (Invitrogen). After transformation into *Escherichia coli* strain BL21(DE3), transformants were cultured in LB broth containing 50 µg/ml ampicillin. When the cultured reached an  $A_{600}$  value of 0.7, isopropyl-β-thiogalactopyranoside (IPTG) was added to the bacterial cultures to a final concentration of 0.5 µM to induce expression. After three hours, the cultures were harvested by centrifugation, and the cells were then sonicated. After extract preparation and analysis by SDS-PAGE, it was found that all of the expressed tag forms of the eEF-2 kinase were in inclusion bodies. Inclusion bodies were precipitated, dissolved in 8.0 M urea, and dialyzed overnight against 20 mM Tris-HCl (ph. 7.0) buffer containing 100 mM NaCl and 4 mM β-mercaptoethanol. The refolded protein was analyzed by SDS-PAGE and assayed for the ability to phosphorylate eEF-2. All of the fusion eEF-2 kinase preparations were able to efficiently phosphorylate eEF-2.

25

### EXAMPLE 9

**eEF-2 Kinase Activity Assay Using a 16-Amino Acid Peptide Derived from Myosin Heavy Chain as the Phosphorylation Target.** It was found that 16'mer peptide, RKKFGESEKTKTKEFL, can serve as a good substrate for eEF-2 kinase. (Note: circular

30

dichroism measurements indicated that this peptide is in an  $\alpha$ -helical structure, and that amidation of the peptide further stabilizes the  $\alpha$ -helical structure, resulting in stronger phosphoacceptor activity.) Since recombinant eEF-2 is impossible to overexpress, as discussed *supra*, and large amounts of the protein are required to for large scale screening assays, the discovery of a peptide (easily synthesized on a large scale) that exhibits the same phosphoacceptor activity as eEF-2 was the critical breakthrough that allows for the development of a variety of automated high throughput screening assays for screening drug candidates.

10 The basic assay is as follows: 0.2-10.0  $\mu$ g of recombinant eEF-2 kinase (produced as described in EXAMPLE 6) is incubated with the 16'mer peptide (described above) in a buffer consisting of 12.5 mM Hepes-KOH (ph. 7.4), 2.5 mM magnesium acetate, 1.25 mM DTT, 25  $\mu$ M  $\text{CaCl}_2$ , 0.05-2.5  $\mu$ g calmodulin, 100  $\mu$ M ATP, and 0.5  $\mu$ Ci  $[\gamma\text{-}^{33}\text{P}]\text{ATP}$  in a total volume of 5-250  $\mu$ l. Samples are incubated at 30°C and aliquots can be  
15 withdrawn at various time points or at a single end point, and the reaction terminated by lowering the temperature ( $\leq 4^\circ\text{C}$ ). The aliquots are then spotted onto phosphocellulose paper (2 cm x 2 cm) and washed (4 x 4 min) with 75 mM phosphoric acid. The papers are then rinsed with 100% ethanol, dried, and then counted in a scintillation counter. The assay can be performed at various peptide concentrations, as was done in the experiment  
20 illustrated in Figure 8. Clearly for a high throughput drug screening assay, that would be amenable to automation, the assays would most likely be performed using one peptide concentration with increasing amounts of different drug (inhibitor) candidates, and the data collected at a single time point. The assay can be performed in any one of the following formats:

- 25 1. with  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$  or  $[\gamma\text{-}^{33}\text{P}]\text{ATP}$  and then detected using either standard scintillation counting, or detected in the format of a homogeneous assay using a Scintillation Proximity Assay, described in detail in both the Amersham Product Catalog (1997), pp. 252-258, and U.S. patent number 4,568,649;

2. in any of a number of standard immunoassay formats using antibodies that are specific for the phosphorylated form of the 16'mer peptide. Detection would then be, as described in more detail *supra*, through the use of either isotopically- or nonisotopically-labeled antibodies, secondary antibodies, or 16'mer peptide.

#### EXAMPLE 10

##### **Cancer cell killing by chemotherapeutic**

##### **drugs requires protein synthesis, and can be blocked by cycloheximide**

10

It has been well established that protein synthesis is required for apoptotic cell death induced by a variety of stimuli, including anticancer drugs. Inhibition of protein synthesis by cycloheximide prevent apoptotic cell death, and protects cancer cells from treatment with anticancer drugs. The exact molecular mechanism by which protein synthesis modulates cell death is unclear. The results demonstrated herein demonstrate that translational elongation factor-2 kinase (eEF-2 kinase) can be activated by a slight decrease in pH. Activation of this kinase leads to commonly observed during apoptotic cell death, activation of this kinase, and hence inhibition of protein synthesis during apoptosis, is expected to be anti-apoptotic. Because of the possible anti-apoptotic function of eEF-2 kinase, inhibition of eEF-2 kinase is expected to sensitize tumors to apoptotic cell death and anticancer drugs. Specifically, activation of eEF-2 kinase in hypoxic solid tumors (a low pH environment) is anti-apoptotic. Consequently, the specific inhibition of eEF-2 kinase can sensitize hypoxic solid tumors in apoptotic cell death and to the cytotoxic action of anticancer drugs.

25

One of the major obstacles in cancer therapy is the resistance of cancer cells to chemotherapeutic drugs. Of the many types of drug resistance, the most commonly addressed is multidrug resistance. Multidrug resistance is a particular phenotype which is characterized by an unusual resistance of cells to a variety of anticancer drugs with unrelated chemical structures. Among various mechanisms of multidrug resistance, the

30

most important and intensively studied is the multidrug resistance conferred by P-glycoprotein. According to the current model, P-glycoprotein protects cells by actively pumping drugs out of cells.

- 5 Recent evidence suggests that there is another type of drug resistance that involves inhibition of programmed cell death, or apoptosis. Due to intensive research in the past several years, it has become well established that anticancer drugs kill cells by inducing apoptosis. Apoptosis is an active process that is accompanied by activation of specific signal transduction pathways, and requires expression of specific proteins. Although  
10 there are a few exceptions, in most cases, it has been observed that inhibition of protein synthesis by cycloheximide can block cell death induced by anticancer drugs. Table 1 provides a list of examples where it was demonstrated that cycloheximide can prevent cytotoxicity of anticancer drugs.

- 15 Table 1: Examples where cycloheximide was shown to protect cells and tissues from cell death caused by anticancer drugs.

Drug	Cell Type	Reference
<b>Topoisomerase I inhibitors</b>		
20 Camptothecin	HL-60 (human promyelocyte)	5. Gong et al. (1993) <i>J. Cell Physiol.</i> <b>157</b> :263-270.
Camptothecin	Mouse thymocytes	6. Onishi et al., (1993) <i>Biochem. Biophys. Acta</i> <b>1175</b> : 147-154.
CPT-11	PLC (human hepatoma)	7. Suzuki & Kato (1996) <i>Exp. Cell Res.</i> <b>227</b> :154-159.
<b>Topoisomerase II inhibitors</b>		
Doxorubicin (adriamycin)	V79 (rodent fibroblasts)	8. Bonner & Lawrence (1989) <i>Int. J. Radiat. Oncol. Biol. Phys.</i> <b>16</b> :1209-1212.

	Doxorubicin (adriamycin)	P388 (mouse leukemia): mice	9. Furusawa et al. (1995) <i>Biol. Pharm. Bull.</i> 18:1367-1372.
	Doxorubicin (adriamycin)	murine intestinal tract; mouse thymocytes	10. Thakkar & Potten (1992) <i>Biochem. Pharm.</i> 43:1683-1691.
	Etoposide	Balb/C 3T3 (mouse fibroblasts); CCRF-CEM (human lymphoblast cells); L1012 (mouse leukemia cells)	11. Chow et al. (1988) <i>Biochem. Pharm.</i> 37:1117-1122.
	Etoposide	mouse thymocytes	12. Sun et al. (1994) <i>Biochem. Pharm.</i> 47: 187-195.
5	<b>Microtubule drugs</b>		
10	Taxol	KB (human epidermoid carcinoma);	13. Ling et al. (1998) <i>Int. J. Cancer</i> 75:925-932
		A549 (human lung adenocarcinoma) MCF-7 (human breast)	14. Liebman et al. (1994) <i>Anticancer Drugs</i> 5:287-292
	Vincristine	Ksu (human osteosarcoma)	15. Sakai et al. (1989) <i>Cancer Res.</i> 49:1193-1196.
	Vincristine	CHO strain AA8	16. Kung et al. (1990) <i>Cancer Res.</i> 50:7307-7317.
	Colchicine	Ksu	15. Sakai et al. (1989) <i>Cancer Res.</i> 49:1193-1196
	<b>Other drugs</b>		
	Ara-C	rat intestine	17. Verbin et al. (1973) <i>Cancer Res.</i> 33:2086-2093.
	Nitrogen mustard	rat intestine	18. Lieberman et al. (1970) <i>Cancer Res.</i> 30:942-951.
	5-AZT	mouse thymocytes	19. Kizaki et al. (1993) <i>Immunopharm.</i> 25:19-27.

Cisplatin	CHO	20. Barry et al. (1990) <i>Biochem. Pharm.</i> 40:2353-2362
Methotrexate	mice	21. Panasci et al. (1982) <i>Cancer Lett.</i> 15:81-86.

- 5 While most of these studies were performed on cells in culture, there is also evidence that protein synthesis is required for the cytotoxic action of anticancer drugs *in vivo*. It was demonstrated by Furusawa et al. (9) that, in mice, the toxicity of doxorubicin as well as its antitumor effects, can be effectively counteracted by injection of cycloheximide. In another study (21) it was found that injection of cycloheximide can
- 10 significantly reduce the toxicity of methotrexate. Thus, inhibition of protein synthesis may be considered a mechanism that confers resistance to anticancer drugs.

**Cellular mechanism of protein synthesis inhibition: elongation factor-2 phosphorylation:** It appears that, although eEF-2 kinase is strictly  $\text{Ca}^{2+}$ /calmodulin-

15 dependant, it requires very low concentrations of  $\text{Ca}^{2+}$ /calmodulin for activity, and physiologically relevant changes of intracellular  $\text{Ca}^{2+}$  are unlikely to cause a strong effect on eEF-2 kinase activity. On the other hand, it appears that changes in pH can drastically modulate eEF-2 kinase (see Preliminary Results). At  $\text{pH} \geq 7.4$ , eEF-2 kinase activity is very low, and increased dramatically 10 to 20-fold upon a slight decrease in

20 pH to 6.6. Thus, eEF-2 kinase appears to be a proton-activated protein kinase. It was also found that the pH-dependance of eEF-2 kinase activity closely correlated with pH dependance of protein synthesis inhibition. This observation suggests that eEF-2 kinase may be responsible for protein synthesis inhibition during acidification of the cytoplasm. Since significant acidification occurs during apoptosis, this acidification

25 may result in the inhibition of protein synthesis due to activation of eEF-2 kinase and phosphorylation of eEF-2. Activation of eEF-2 kinase by acidic pH may explain the previously reported strong increase in eEF-2 phosphorylation seen in brain tissue from



Alzheimer's patients, as well as that seen in neurons after treatment with glutamate (35, 36).

**The role of eEF-2 kinase in drug resistance:** Activation of eEF-2 kinase by an acidic pH suggests that it may play an important role in regulation of protein synthesis during apoptosis. It is now well established that a significant decrease in intracellular pH is part of the apoptotic process. A drop in pH by 0.5-1 pH unit is universally observed during apoptosis induced by various agents, including anticancer drugs (37-43). It was found that at an intracellular pH of 6.5-6.8, eEF-2 becomes strongly phosphorylated. This phosphorylation of eEF-2 at an acidic pH may explain the inhibition of protein synthesis that was observed during apoptosis (44, 45).

Since apoptosis requires ongoing protein synthesis, phosphorylation of eEF-2, and the resulting inhibition of translation, may be an anti-apoptotic mechanism. Thus, it is suggested that eEF-2 phosphorylation is a cellular mechanism that can protect cells from apoptosis. To test this hypothesis, the effect of overexpression of eEF-2 kinase on drug resistance of mouse fibroblasts was tested. As is shown, overexpression of eEF-2 kinase increases approximately 10-fold the resistance of cells to different cytotoxic drugs, namely camptothecin and teniposide. This result suggests that eEF-2 kinase may be involved in modulation of drug resistance. Thus, inhibition of protein synthesis mediated by eEF-2 phosphorylation can protect cells from apoptotic cell death by various mechanisms that include up-and downregulation of expression of many genes. The pH-dependant increase in eEF-2 phosphorylation may also explain the previously observed dramatic increase in malignant cell resistance at low pH. It was demonstrated that at pH 6.5-6.8, different cell lines became more resistant to mitoxantrone, paclitaxel, and topotecan (46, 47).

Drug resistance related to activation of eEF-2 kinase can be particularly important in the hypoxic interior of solid tumors, which are characterized by acidic pH (48, 49). In fact, it was recently demonstrated that eEF-2 kinase activity is drastically

upregulated in tumor cell lines (161) and in invasive breast cancer specimens obtained from patients (162). Thus, eEF-2 kinase can be an important mechanism contributing to drug resistance of cancer cells.

5

Accordingly, specific inhibition of eEF-2 kinase can sensitize cancer cells to apoptotic cell death and to the cytotoxic action of anticancer drugs.

The results demonstrate that eEF-2 kinase was dramatically activated by a decrease in pH within the range that occurs during apoptosis and that the pH-dependence of eEF-2 kinase activation correlated with protein synthesis inhibition *in vivo* and that overexpression of eEF-2 kinase in mouse fibroblasts increased their resistance to cytotoxic drugs. Using deletion mutagenesis, it was determined the tentative location of the various functional domains of eEF-2 kinase.

15

**Activation of eEF-2 kinase by low pH:** eEF-2 kinase is a  $\text{Ca}^{2+}$ /calmodulin-dependant enzyme whose only known substrate is eEF-2. Initially, it was suggested that the function of eEF-2 kinase was to phosphorylate eEF-2, and arrest protein synthesis in response to an elevation of  $\text{Ca}^{2+}$  levels in the cytoplasm. However, in a recent detailed study of the relationship between intracellular  $\text{Ca}^{2+}$  levels, eEF-2 phosphorylation, and protein synthesis in GH3 cells, it was shown that a superphysiological increase in  $\text{Ca}^{2+}$  levels in the cytoplasm produced only an insignificant increase in eEF-2 phosphorylation that did not affect translation (34).

25 Analysis of the pH dependence of recombinant GST-eEF-2 kinase demonstrated that both autophosphorylation activity and eEF-2 phosphorylation activity were markedly pH-dependent. The pH-dependence of eEF-2 kinase activity was quantitatively analyzed using a synthetic 16mer peptide substrate (MH-1; RKKFGESEKTKTKEFL-amide. MH-1 corresponds to the MHCK A phosphorylation site in *Dictyostelium* myosin heavy chains. It was found it to be an efficient substrate for eEF-2 kinase.

30

Activity of GST-eEF-2 kinase was assayed as follows: Purified GST-eEF-2 kinase was incubated with MH-1 (100  $\mu$ M final) in a buffer consisting of 25 mM Hepes-KOH (pH varying), 5 mM magnesium acetate, 2.5 mM DTT, 50  $\mu$ M  $\text{CaCl}_2$ , 0.5  $\mu$ g calmodulin, 100  $\mu$ M ATP, and 0.5  $\mu$ Ci [ $\gamma$ - $^{33}\text{P}$ ]-ATP (specific activity = 2000 Ci/mmol). The total  
5 volume of the reaction was 50  $\mu$ l. The reaction was run at 30°C for various periods of time, and was terminated by incubation in an ice-water bath. An aliquot of each reaction was spotted onto a 2cm x 2 cm square of phosphocellulose paper and then washed 4 x 4 minutes in 75 mM phosphoric acid. After a 30 second rinse in 100% ethanol, the filter papers were dried, and then counted in a scintillation counter. To  
10 assay for autophosphorylation activity, kinase assays were run as above except that the peptide was omitted from the reaction mixture.

Figure 10 shows that phosphorylation of the peptide is very ineffective at pH  $\geq 7.4$ , but when the pH drops to 6.6, eEF-2 kinase becomes dramatically activated. It was also  
15 analyzed how calmodulin activates eEF-2 kinase at different pH. eEF-2 kinase assays were done as described above, but with different concentrations of calmodulin at pH 6.6 and at pH 7.4. It appears that at pH 6.6, the  $K_m$  for calmodulin is about  $10^9$  M, while at pH 7.4, the  $K_m$  is approximately  $10^7$ . These results suggest that changes in intracellular  $\text{Ca}^{2+}$  at physiological pH-will not significantly affect eEF-2 kinase activity,  
20 which is consistent with recently reported results on GH3 cells (34).

To determine how specific this pH effect is for eEF-2 kinase, the pattern of protein phosphorylation at different pH in extracts from rat heart tissue was analyzed. A heart was dissected from a freshly killed rat and frozen in liquid nitrogen. The frozen tissue  
25 was homogenized in a buffer containing 25 mM Hepes-KOH (pH 7.4), 100 mM NaCl, 3mM EDTA, 2mM EGTA, 40  $\mu$ g/ml soybean trypsin inhibitor, 0.5 mM PMSF, 20 mM Na pyrophosphate. The homogenate was clarified by centrifugation for 20 min. at 16,000 xg at 4°C. In order to assay for eEF-2 kinase activity, 10  $\mu$ l of the homogenate was added to a reaction mixture containing 50 mM Hepes-KOH (at various  
30 pH), 10 mM magnesium acetate, 5 mM DTT, 100  $\mu$ M  $\text{CaCl}_2$ , 0.5  $\mu$ g calmodulin, 60

$\mu$ M ATP, and 2  $\mu$ Ci [ $\gamma$ - $^{33}$ P]-ATP (specific activity = 2000 Ci/mmol). The total volume of the reaction was 40  $\mu$ l. The reaction was run at 30°C for 5 minutes, and was terminated by incubation in an ice-water bath. Laemmli sample buffer was added, and the reaction mixture was boiled for 5 minutes. Samples were analyzed by 8% SDS-PAGE and autoradiography. As can be seen from Figure 3, eEF-2 was the only protein

5 whose phosphorylation increased in response to a decrease in pH.

Next whether a decrease in  $pH_i$  of cells in culture resulted in inhibition of protein synthesis, and whether it correlated with activation of eEF-2 kinase were analyzed.

10 Protein synthesis was measured in GH3 cells by [ $^3$ H]-Leu pulse-incorporation. GH3 cells were pre-incubated for 30 minutes in Ham's F-10 medium at 0.2 pH unit intervals from pH 6.0 to 8.0. Leucine pulse-incorporation was measured as described in Brostrom et al. (115). 1 mM  $Ca^{2+}$  and 100  $\mu$ M [ $^3$ H]-Leu were added to the medium for a 15 minute incorporation period. Cells were harvested by centrifugation, washed,

15 and lysed. Unincorporated label was removed by TCA precipitation, and Leu incorporation was measured by scintillation counting.

The actual intracellular pH was verified by incorporating BCECF/AM into GH3 cells in balanced salt solution buffered at pH 7.4 for 30 minutes. One set of cells was

20 washed and re-suspended in normal balanced salt solutions at 0.2 pH unit intervals between 6.0 and 7.8. The other set of cells was re-suspended in high-K + buffer containing nigericin. A ratio of excitation of 485nm/440nm with emission at 530 nm was determined for each sample and  $pH_i$  was calculated as described by Thomas et al. (116).

25

The results of these experiments are shown in Figure 11. A decrease in the intracellular pH produced a strong inhibition of protein synthesis, which indeed correlated with the pH-dependence of eEF-2 kinase activity. These results demonstrate that eEF-2 phosphorylation may be a mechanism responsible for inhibiting protein

30 synthesis at a low intracellular pH. Since the decrease in intracellular pH to the level

that strongly activates eEF-2 kinase is universally observed during apoptosis (37-43), this provides evidence that eEF-2 phosphorylation may be responsible for protein synthesis inhibition during apoptosis.

- 5 **Effect of overexpression of eEF-2 kinase on the cellular resistance to anticancer drugs:** Cell lines overexpressing eEF-2 kinase by stably transfecting N1H/3T3 cells with full length murine eEF-2 kinase cDNA were created. Murine eEF-2 kinase cDNA was cloned into pCMV-SPORT2 (GIBCO/BRL) under the control of a CMV promoter. N1H/3T3 cells were co-transfected with pCMV-SPORT2-EF2K and pSV2neo, using  
10 Lipofectamine (GIBCO/BRL). Stable transformants were selected with G<sub>418</sub> (0.5 mg/ml), and were further maintained in medium containing G<sub>418</sub>. A control cell line (C13) was produced by co-transfection of N1H/3T3 cells with empty pCMV-SPORT2/pSV2neo. The expression of eEF-2 kinase mRNA in the selectants was assayed by Northern blot analysis. Two cloned cell lines, designated as E8 and E9  
15 were found to overexpress significantly eEF-2 kinase mRNA, with E9 giving the maximal level of expression.

In order to verify that overexpression of eEF-2 kinase mRNA in E8 and E9 produced an increase in eEF-2 kinase activity, cell lysates were assayed for the ability to  
20 phosphorylate eEF-2. eEF-2 kinase assays were performed as described above. E8 and E9 indeed showed greatly increased eEF-2 kinase activity in comparison to control cells. Overexpression of eEF-2 kinase did not have any effect on the growth parameters of cells. Cell cycle distribution of exponentially growing or quiescent cells, as well as growth rates, were the same for clones 8, 9 and control clones.

25

To answer the question whether eEF-2 kinase plays a role in resistance, clone E9 overexpressing eEF-2 kinase was incubated with two cytotoxic drugs, and assessed cell survival by MTT cytotoxicity assay. E9 and C13 were plated in 96-well plates at 3000 cells per well. Cells were grown in DMEM with 10% FCS for 1 day, and then  
30 incubated with different concentrations of camptothecin (CPT) or teniposide (VM26)

for 4 days, and MTT assays were performed (Figure 12). E9 showed significantly increased resistance to these drugs.

### EXAMPLE 11

#### 5           **Expression and mutagenesis of recombinant human eEF2 kinase.**

Methods for efficient expression of recombinant eEF-2 kinase in bacteria, as well as generated and analyzed thirteen deletion mutants were developed. Expression of wild-type 6xHis-tagged and GST-tagged human eEF-2 kinase in *E. coli*. Initially, human eEF-  
10 2 kinase was expressed as a fusion protein with 6xHis or glutathione-S-transferase (GST). Human eEF-2 kinase cDNA was cloned into two different expression vectors: pRSET (Invitrogen). and pGEX-2T (Pharmacia). After the resulting vectors were transformed into *E. coli* strain JM109(DE3), the transformants were cultured in LB broth containing 50 mg/ml ampicillin. At log phase growth, isopropyl-b-thiogalactopyranoside  
15 (IPTG) was added to the bacterial cultures to a final concentration of 0.5 mM. After three hours, the cultures were harvested and the cells were sonicated.

Both 6xHis-tagged and GST-tagged eEF-2 kinase were efficiently expressed as judged by the appearance of one major band on Coomassie-stained gels after SDS-PAGE  
20 analysis of crude lysates. Therefore, eEF-2 kinase activity was analyzed directly in the crude lysates. In order to assay for eEF-2 kinase activity, bacterial lysates were incubated with purified rabbit reticulocyte eEF-2 (0.5 mg) in a buffer consisting of 50 mM Hepes-KOH (pH 7.4), 10 mM magnesium acetate, 5 mM DTT, 100 mM CaCl<sub>2</sub>, 0.5 mg calmodulin, 60 mM ATP, and 2 mCi [γ-<sup>32</sup>P]-ATP (specific activity = 2000 Ci/mmol).  
25 The total volume of the reaction was 40 ml. The reaction was run at 30°C for 10 minutes, and was terminated by incubation in an ice-water bath. Laemmli sample buffer was added, and the reaction mixture was boiled for 5 minutes. Samples were analyzed by 8% SDS-PAGE. The gel was dried and exposed to film overnight.



As can be seen in Figure 13, recombinant eEF-2 kinase expressed in *E. coli* undergoes autophosphorylation upon incubation with [ $\gamma$ - $^{33}\text{P}$ ]-ATP and can efficiently phosphorylate eEF-2. The ability of eEF-2 kinase to undergo autophosphorylation was previously reported for the enzyme purified from rabbit reticulocyte lysate (139).

5 Autophosphorylated eEF-2 kinase was represented by two or three distinct bands, showing that autophosphorylation of eEF-2 kinase occurs at multiple sites, and slightly affects its mobility in the gel. Both the ability of eEF-2 kinase to autophosphorylate and phosphorylate eEF-2 were strictly calmodulin-dependent (see Figure 13).

10 **Localization of eEF-2 kinase functional domains by *in vitro* mutagenesis:** Using *in vitro* mutagenesis, thirteen mutants of eEF-2 kinase were obtained with deletions ranging from 36 to 76 amino acids that systematically span the entire

**eEF-2 kinase molecule:** *In vitro* mutagenesis was done with the Muta-Gene Phagemid  
15 *In Vitro* Mutagenesis kit from Bio-Rad which is based on a method developed by Kunkel (117). Human eEF-2 kinase cDNA was cloned into pCR2.1 (Invitrogen). This plasmid has an f1 ori so that it can exist as single-stranded, as well as double-stranded DNA. It also carries the genes for ampicillin and kanamycin resistance. Thus, this plasmid was found to be suitable for use with the Muta-Gene kit.

20

The construct was transformed into *E. coli* strain CJ236, a *dut ung* strain. The enzymes dUTPase and uracil-N-glycosylase have been mutated and are non-functional, thus allowing for a large pool of uracil to be maintained in the cell as well as to allow uracil to be incorporated into the replicated plasmid. Bacteria carrying the plasmid were  
25 selected by growth in medium containing ampicillin. In order to produce single-stranded DNA, cultures of CJ236 were infected with helper phage M13K07. Replication of the M13K07 genome has been partly disabled and, thus, the low copy number of the M13K07 genome allows for more packaging of the plasmid instead of helper phage. This minimizes contamination of the harvested single-stranded DNA with M13K07 DNA.  
30 There is a kanamycin resistance marker in the M13K07 genome which allows for



selection of infected bacteria by growing them in the presence of kanamycin. After purification from the helper phage, the single-stranded DNA was mutagenized. 30-mer oligonucleotides were synthesized which were complementary to a stretch of fifteen nucleotides on each side of the region to be deleted. Annealing of the oligo to single-stranded DNA caused the region to be deleted to loop out. The oligo acted as a primer for second-strand synthesis by T7 DNA polymerase. Synthesis was done in the presence of thymidine so that the resulting double-stranded construct was a hybrid of a uracil-containing strand and a thymidine-containing strand. The mutant plasmid was then transformed into *E. coli* strain DH5a. This strain has a functional uracil-N-glycosylase which inactivates the uracil-containing strand and allows the thymidine-containing strand to be replicated. After purification, the mutant plasmid was sequenced to verify that the proper deletion was made. Initially, the resulting thirteen mutants were expressed *in vitro* in a coupled transcription/translation system and assayed for eEF-2 kinase activity.

Although using an *in vitro* transcription/translation system is fast and efficient, there are two disadvantages. First, it is not quantitative. Second, the amount of protein expressed *in vitro* is very low. To overcome this problem, the thirteen mutants were cloned into pGEX-2T (Pharmacia), and expressed as GST fusion proteins in *E. coli*.

Expression of GST-eEF-2 kinase deletion mutants in bacteria and purification. All thirteen mutants were expressed in *E. coli* BL21(DE3) as GST-tagged proteins. Expression of GST-tagged human eEF-2 kinase was done as follows: the cDNA for the mutant forms human eEF-2 kinase was cloned into pGEX-2T, and transformed into *E. coli* BL21(DE3). Cultures were grown at 37°C, and at log phase growth, protein expression was induced with IPTG added to a final concentration of 0.5 mM. After a 3 hour incubation at 37°C with IPTG, cells were harvested by centrifugation.

Since wild-type and mutant eEF-2 kinase were found to be highly insoluble, the proteins were purified from inclusion bodies. Bacterial cells were re-suspended in sonication buffer (phosphate-buffered saline, 1 mg/ml lysozyme, 3 mM EDTA, 40 mg/ml soybean

trypsin inhibitor, 0.5 mM PMSF, 20 mM Na pyrophosphate) and sonicated. Lysate was centrifuged at 16,000xg for 30 minutes at 4°C to pellet inclusion bodies. Pellet was re-suspended in a buffer containing 20 mM Tris-HCl (pH 7.0), 100 mM NaCl, 7 mM b-mercaptoethanol, 6 M urea and incubated on ice for 20 minutes. Lysate was dialyzed  
5 overnight at 4°C against buffer consisting of 20 mM Tris-HCl (pH 7.0), 100 mM NaCl, and 7 mM b-mercaptoethanol. Dialyzed material was centrifuged at 16,000 xg for 20 min. at 4°C to remove any remaining insoluble material.

Glutathione-agarose (Sigma) was added to the dialysate (bed volume = 1/2000 of culture  
10 volume), and incubated at 4°C for 2 hours with gentle shaking. Glutathione agarose was pelleted by centrifugation and washed 3x with PBS (10x bed volume). Bound GST-EF-2 kinase was eluted with 50 mM Tris-HCl (pH 8.0) containing 25 mM reduced glutathione (Volume of elution buffer = bed volume). Elution was done on ice for 20 minutes.

15 **Analysis of activity of deletion mutants:** The thirteen deletion mutants were assayed for the ability to phosphorylate eEF-2 and to undergo autophosphorylation. eEF-2 (Figure 14)kinase assays were performed as described above using purified rabbit reticulocyte eEF-2 as a substrate, and the pH of the reaction was 6.4. Mutants with deletions between amino acids 51-335 were neither able to phosphorylate eEF-2 nor to  
20 undergo autophosphorylation. On the other hand, deletions between amino acids 521-725 caused a loss of eEF-2 kinase activity, but not a loss of autophosphorylation activity. This demonstrates that the catalytic domain is located between amino acids 51-335 while the region between amino acids 521-725 is important for eEF-2 recognition. The region between amino acids 336-520 probably serves as a hinge between two domains.

25

A summary of the results of mutational analysis of human eEF-2 kinase is shown in Figure 14. Mutational analysis reveals that eEF-2 kinase can be subdivided into two domains connected by a hinge region. The N-terminal domain represents the catalytic domain, and the C-terminal domain represents the eEF-2 targeting domain. This is  
30 consistent with the location of the catalytic domain as predicted by comparison of eEF-2

kinases from different species (27). As was discussed in the Background and Significance section, the amino acid sequence of this region displays no homology to the catalytic domains of the conventional protein kinases, but appears to be highly similar to the catalytic domains of the recently described myosin heavy chain kinases from  
5 *Dictyostelium*. Further experiments will define more precisely the location of the functional domains.

The results clearly demonstrate that the location of the catalytic domain of rat eEF-2 kinase suggested by Redpath *et al.* (140) in between amino acids 288-554 is incorrect.  
10

**Location of calmodulin-binding domain:** In all mutants able to undergo autophosphorylation, this phosphorylation was strictly calcium/ calmodulin-dependent, suggesting that the calmodulin-binding region is located within amino acids 51 to 335.  
15 To locate the calmodulin-binding domain more precisely, five mutants were analyzed with deletions between amino acids 51 to 355 for their ability to bind calmodulin-agarose. The only mutant of eEF-2 kinase not able to bind calmodulin-agarose contains a deletion of amino acids 51-96. The actual calmodulin-binding site is probably within amino acids 81 to 94 (FKEAWKHAIQKAKH), which are predicted to form an  
20 amphipathic  $\alpha$ -helix. The homologous sequence from *C. elegans* eEF-2 kinase (LMETWRKAARRART) is also predicted to form an amphipathic  $\alpha$ -helix.

**Expression of correctly-folded eEF-2 kinase:** Although GST- and 6xHis-eEF-2 kinase expressed in bacteria was enzymatically active, the majority of recombinant protein  
25 ended up in inclusion bodies. The inclusion bodies were precipitated, dissolved in 8M urea, and dialyzed overnight against 20 mM Tris buffer (pH 7.0) containing 100 mM NaCl and 4 mM  $\beta$ -mercaptoethanol. The refolded protein was analyzed by SDS-PAGE and assayed for the ability to undergo autophosphorylation and to phosphorylate eEF-2. Preparations of refolded GST-eEF-2 kinase and 6xHis-eEF-2 kinase contained  
30 predominantly one band corresponding to eEF-2 kinase. In the case of GST-tagged eEF-

2 kinase, the protein was further purified using glutathione-Sepharose. Gel filtration analysis of recombinant eEF-2 kinase on Superdex-200 revealed that both 6xHis- and GST-eEF-2 kinase eluted predominantly in the void volume, indicating that it was aggregated. This aggregated kinase was enzymatically inactive. eEF-2 kinase activity  
5 eluted in the fractions corresponding to a Stokes radius of 52. Approximately 4% of the refolded eEF-2 kinase eluted in this area. The specific activity of eEF-2 kinase was 5 mmoles/min/mg, which is slightly higher than the specific activity of eEF-2 kinase purified from rabbit reticulocytes. The extremely low yield of active eEF-2 kinase prompted to search for alternative methods for expression of recombinant kinase.

10

eEF-2 kinase was expressed as fusion with thioredoxin in order to obtain a soluble protein because it was reported that thioredoxin can prevent accumulation of recombinant proteins in inclusion bodies (149). Human eEF-2 kinase cDNA was cloned into the expression vector pThioHis (Invitrogen). The thioredoxin expressed from this vector has  
15 additional histidine residues so it can also function as a His tag. Thioredoxin-eEF-2 kinase was found to remain soluble and was enzymatically active. Moreover, its elution profile on Mono Q and Superdex-200 was very similar to eEF-2 kinase purified from rabbit reticulocytes, which shows that it was correctly folded. This His-thioredoxin-tagged kinase can be efficiently and easily purified from total lysates by immobilized  
20 metal affinity chromatography (IMAC) or by ThioBond resin (Invitrogen). This fusion protein also contains an enterokinase cleavage site, which allows for removal of the His-thioredoxin tag. Thus, a method to express active and, most likely correctly folded, eEF-2 kinase which will facilitate the studies was demonstrated.

25 **Phosphorylation of synthetic peptides by eEF-2 kinase:** Redpath *et al.* (118) were able to detect some [ $\gamma$ - $^{32}\text{P}$ ]-ATP incorporation into a synthetic peptide corresponding to residues 49-60 of eEF-2 (RAGETRFTDTRK), which encompasses the phosphorylation site of eEF-2. The rate of phosphorylation was low, and the  $K_m$  was very high ( $\gg 2.5$  mM). A larger peptide corresponding to amino acids 48-66 of eEF-2

(ARAGETRFTDTRKDEQERC), was synthesized and was unable to detect any phosphorylation after incubation with [ $\gamma$ - $^{33}\text{P}$ ]-ATP and recombinant eEF-2 kinase.

As can be seen in Figure 15, eEF-2 kinase can efficiently phosphorylate MH-1. The  $K_m$  of this reaction was approximately 150 mM, which is similar to the  $K_m$  of the phosphorylation of MH-1 by MHCK A (105 mM; 119). If an  $\alpha$ -helical conformation is necessary for recognition of MH-1 by eEF-2 kinase, then removal of the C-terminal amide from MH-1 should reduce the  $\alpha$ -helical propensity of the peptide, and thus, make it a less efficient substrate for eEF-2 kinase. As can be seen in Figure 11a, this is the case. An uncapped version of the peptide (called MH-U) can serve as a substrate for eEF-2 kinase, although a significantly less efficient one. The reduction of phosphorylation efficiency is due entirely to a change in  $K_m$ , which for MH-U is approximately 400 mM.

It is interesting that the sequence of MH-1 is quite different from the sequence surrounding the phosphorylation site in eEF-2 (see Figure 15B). When looking at the primary structure, the threonines that undergo phosphorylation are surrounded in these two peptides by very different amino acids. But when the same sequences are folded into  $\alpha$ -helices, the surrounding environment of those phosphoacceptor threonines is very similar, as can be seen in Figure 15B: in both cases, there is a basic amino acid to the left and a glutamate followed by a basic amino acid on the right. This pattern may represent the consensus sequence for recognition by eEF-2 kinase and the related protein kinases. Thus, as demonstrated herein eEF-2 kinase phosphorylates amino acids located within  $\alpha$ -helices.

25

While the invention has been described and illustrated herein by references to various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art.

30

WHAT IS CLAIMED IS:

- 1    1.    A protein kinase which is a member of a superfamily, said protein kinase  
2    being characterized by:
  - 3                    A.    greater than 40% sequence similarity with eEF-2 kinase from  
4    any organism; and,
  - 5                    B.    phosphorylates an amino acid within an alpha helical domain  
6    of its target protein.
- 1    2.    A protein kinase of Claim 1 which phosphorylates eukaryotic elongation  
2    factor-2 (eEF-2), and is designated as eukaryotic elongation factor-2 kinase (eEF-2  
3    kinase).
- 1    3.    A protein kinase of Claim 1 which phosphorylates eukaryotic myosin heavy  
2    chain (MHC), and is designated as myosin heavy chain kinase (MHCK).
- 1    4.    A protein kinase of Claim 1 that phosphorylates a peptide sequence derived  
2    from the phosphorylation site of a target protein.
- 1    5.    A peptide sequence having SEQ ID NO: 20.
- 1    6.    A protein kinase of Claim 1 which is a polypeptide having an amino acid  
2    sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and  
3    SEQ ID NO: 10, and fragments thereof.
- 1    7.    A protein kinase of Claim 1 which is derived from mammalian cells.
- 1    8.    A protein kinase of Claim 1 labeled with a detectable label.

- 1 9. A protein kinase of Claim 8 wherein the label is selected from enzymes,  
2 chemicals which fluoresce, and radioactive elements.
- 1 10. An antibody to the protein kinase of Claim 1.
- 1 11. An antibody to the phosphorylated form of the target protein of Claim 1.
12. An antibody to the phosphorylated form of the peptide of Claim 5.
- 1 13. The antibody of Claim 10, 11 or 12 which is a polyclonal antibody.
- 1 14. The antibody of Claim 10, 11 or 12 which is a monoclonal antibody.
- 1 15. An immortal cell line that produces a monoclonal antibody according to  
2 Claim 14.
- 1 16. The antibody of Claim 10, 11 or 12 labeled with a detectable label.
- 1 17. The antibody of Claim 10, 11 or 12 wherein the label is selected from  
2 enzymes, chemicals which fluoresce and radioactive elements.
- 1 18. A DNA sequence which encodes eEF-2 kinase, or a fragment thereof,  
2 selected from the group consisting of:
- 3 (A) the DNA sequences of Figure 5 (SEQ ID NO: 1);  
4 (B) the DNA sequences of Figure 5 (SEQ ID NO: 3);  
5 (C) the DNA sequences of Figure 5 (SEQ ID NO: 9);  
6 (D) DNA sequences that hybridize to any of the foregoing DNA  
7 sequences under standard hybridization conditions;  
8 (E) DNA sequences that code for expression of an amino acid sequence  
9 encoded by any of the foregoing DNA sequences.



- 10 (F) degenerate variants thereof;  
11 (G) alleles thereof; and,  
12 (H) hybridizable fragments thereof.

1 19. A recombinant DNA molecule comprising a DNA sequence which encodes  
2 eEF-2 kinase, or a fragment thereof, selected from the group consisting of:

- 3 (A) the DNA sequences of Figure 5 (SEQ ID NO: 1);  
4 (B) the DNA sequences of Figure 5 (SEQ ID NO: 3);  
5 (C) the DNA sequences of Figure 5 (SEQ ID NO: 9);  
6 (D) DNA sequences that hybridize to any of the foregoing DNA  
7 sequences under standard hybridization conditions;  
8 (E) DNA sequences that code for expression of an amino acid sequence  
9 encoded by any of the foregoing DNA sequences.  
10 (F) degenerate variants thereof;  
11 (G) alleles thereof; and,  
12 (H) hybridizable fragments thereof.

1 20. The recombinant DNA molecule of either of Claims 18 or 19, wherein said  
2 DNA sequence is operatively linked to an expression control sequence.

1 21. The recombinant DNA molecule of Claim 20, wherein said expression  
2 control sequence is selected from the group consisting of the early or late promoters  
3 of SV40 or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC*  
4 system, the major operator and promoter regions of phage  $\lambda$ , the control regions of  
5 fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid  
6 phosphatase and the promoters of the yeast  $\alpha$ -mating factors.

1 22. A probe capable of screening for eEF-2 kinase in alternate species prepared  
2 from the DNA sequence of Claim 18.

1 23. A probe capable of screening for members of the protein kinase superfamily  
2 of Claim 1 prepared from the DNA sequence of Claim 18.

1 24. A unicellular host transformed with a recombinant DNA molecule  
2 comprising a DNA sequence or degenerate variant thereof, which encodes a protein  
3 kinase, or a fragment thereof, selected from the group consisting of:

4 (A) the DNA sequences of Figure 5 (SEQ ID NO: 1);

5 (B) the DNA sequences of Figure 5 (SEQ ID NO: 3);

6 (C) the DNA sequences of Figure 5 (SEQ ID NO: 9);

7 (D) DNA sequences that hybridize to any of the foregoing DNA  
8 sequences under standard hybridization conditions; and

9 (E) DNA sequences that code on expression for an amino acid sequence  
10 encoded by any of the foregoing DNA sequences;

11 wherein said DNA sequence is operatively linked to an expression control  
12 sequence.

1 25. The unicellular host of Claim 24 wherein the unicellular host is selected from  
2 the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, yeasts, CHO,  
3 R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells, plant cells,  
4 insect cells, and human cells in tissue culture.

1 26. A method for detecting eEF-2 kinase and assessing eEF-2 kinase levels by:

2 A. contacting a biological sample from a mammal in which the  
3 presence or activity of said eEF-2 kinase is suspected with a binding partner of said  
4 eEF-2 kinase under conditions that allow binding of said eEF-2 kinase to said  
5 binding partner to occur; and,

6 B. detecting whether binding has occurred, and to what degree,  
7 between said eEF-2 kinase from said sample and the binding partner;

8            wherein the detection of binding indicates that presence or activity of said  
9 eEF-2 kinase in said sample, and indicates a level of said eEF-2 kinase in the  
10 sample.

1    27.    An assay system for screening drugs and other agents for ability to modulate  
2 eEF-2 kinase activity, comprising a predetermined amount of eEF-2 kinase mixed  
3 with varying amounts of drug or agent, along with target protein and ATP; wherein  
4 detection is *via* either a detectable label on the  $\gamma$ -phosphate of ATP, or on an  
5 antibody directed against the phosphorylated target protein..

1    28.    The assay system of Claim 27 wherein the label on the  $\gamma$ -phosphate of ATP  
2 comprises one of the following:

- 3            A.     $^{32}\text{P}$ ;
- 4            B.     $^{33}\text{P}$
- 5            C.     $^{35}\text{S}$
- 6            D.    a biotinylated phosphate moiety; or,
- 7            E.    a fluorescent phosphate moiety.

1    29.    The assay system of Claim 27 wherein the label on the antibody comprises  
2 one of the following:

- 3            A.    an enzyme detectable with colorimetric, fluorescent, or  
4 chemiluminescent substrates, such as alkaline phosphatase or horseradish  
5 peroxidase;
- 6            B.    a biotin moiety;
- 7            C.    a fluorescent moiety; or,
- 8            D.    a radioactive moiety chosen from the following group of  
9 isotopes:  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^{36}\text{Cl}$ ,  $^{51}\text{Cr}$ ,  $^{57}\text{Co}$ ,  $^{58}\text{Co}$ ,  $^{59}\text{Fe}$ ,  $^{90}\text{Y}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ , and  $^{186}\text{Re}$ .

1    30.    An assay system for screening drugs and other agents for ability to modulate  
2 eEF-2 kinase activity, comprising:

- 3                   A.     culturing an observable cellular test colony inoculated with a  
4 drug or agent;  
5                   B.     harvesting a supernatant from said cellular test colony; and,  
6                   C.     examining said supernatant for the presence of said eEF-2  
7 kinase activity wherein an increase or a decrease in a level of said eEF-2 kinase  
8 activity indicates the ability of a drug to modulate the activity of said eEF-2 kinase.

1   31.    A test kit for assessing the level of eEF-2 kinase activity in a eukaryotic  
2 cellular sample, comprising:

- 3                   A.   a predetermined amount of a detectably labelled specific binding  
4 partner of eEF-2 kinase.  
5                   B.   other reagents; and,  
6                   C.   directions for use of said kit.

1   31.    The test kit of Claim 31 wherein said labeled immunochemically reactive  
2 component is selected from the group consisting of polyclonal antibodies to eEF-2  
3 kinase, monoclonal antibodies to eEF-2 kinase, fragments thereof, and mixtures  
4 thereof.

1   32.    A method of preventing and/or treating cellular debilitations, derangements  
2 and/or dysfunctions and/or other disease states in mammals, comprising  
3 administering to a mammal a therapeutically effective amount of a material selected  
4 from the following group:

- 5                   A.   peptides that inhibit eEF-2 kinase;  
6                   B.   antibodies against eEF-2 kinase; and,  
7                   C.   other drugs or agents that specifically inhibit eEF-2 kinase.

1   33.    A pharmaceutical composition for the treatment of cellular debilitation,  
2 derangement and/or dysfunction in mammals, comprising:

- 3                   A. a therapeutically effective amount of a material selected from the  
4 group consisting of: peptides that inhibit eEF-2 kinase; antibodies against eEF-2  
5 kinase; and, other drugs or agents that specifically inhibit eEF-2 kinase; and,  
6                   B. a pharmaceutically acceptable carrier.

1   34.    A recombinant virus transformed with the DNA molecule, or a derivative or  
2 fragment thereof, in accordance with Claim 18.

1   35.    A recombinant virus transformed with the DNA molecule, or a derivative or  
2 fragment thereof, in accordance with Claim 19.

1   36.    The recombinant DNA molecule of Claim 20 comprising plasmid pGEX-3X,  
2 clone E3 or plasmid pGEX-3X, clone E4.

1   37.    An antisense nucleic acid against eEF-2 kinase mRNA comprising a nucleic  
2 acid sequence hybridizing to said mRNA.

1   38.    The antisense nucleic acid of Claim 37 which is RNA.

1   39.    The antisense nucleic acid of Claim 37 which is DNA.

1   40.    The antisense nucleic acid of Claim 37 which binds to the initiation codon of  
2 any of said mRNAs.

1   41.    A recombinant DNA molecule having a DNA sequence which, on  
2 transcription, produces an antisense ribonucleic acid against eEF-2 kinase mRNA,  
3 said antisense ribonucleic acid comprising an nucleic acid sequence capable of  
4 hybridizing to said mRNA.

1 42. A eEF-2 kinase-producing cell line transfected with the recombinant DNA  
2 molecule of Claim 41.

1 43. A method for creating a cell line which exhibits reduced expression of eEF-  
2 kinase, comprising transfecting a eEF-2 kinase-producing cell line with a  
3 recombinant DNA molecule of claim 41.

1 44. A ribozyme that cleaves eEF-2 kinase mRNA.

1 45. The ribozyme of Claim 44 which is a *Tetrahymena*-type ribozyme.

1 46. The ribozyme of Claim 44 which is a Hammerhead-type ribozyme.

1 47. A recombinant DNA molecule having a DNA sequence which, upon  
2 transcription, produces the ribozyme of claim 44.

1 48. A eEF-2 kinase-producing cell line transfected with the recombinant DNA  
2 molecule of claim 47.

1 49. A method for creating a cell line which exhibits reduced expression of eEF-2  
2 kinase, comprising transfecting a eEF-2 kinase-producing cell line with the  
3 recombinant DNA molecule of claim 44.

1 50. An isolated nucleic acid encoding a protein kinase which is a member of a  
2 superfamily, said protein kinase being characterized by: A. greater than 40%  
3 sequence similarity with eEF-2 kinase from any organism; and, B. phosphorylates an  
4 amino acid within an alpha helical domain of its target protein.

- 1 51. The isolated nucleic acid of claim 50, wherein the nucleic acid encodes eEF-
- 2 2 kinase protein, heart protein kinase, melanoma protein protein, or ch4 protien
- 3 kinase.



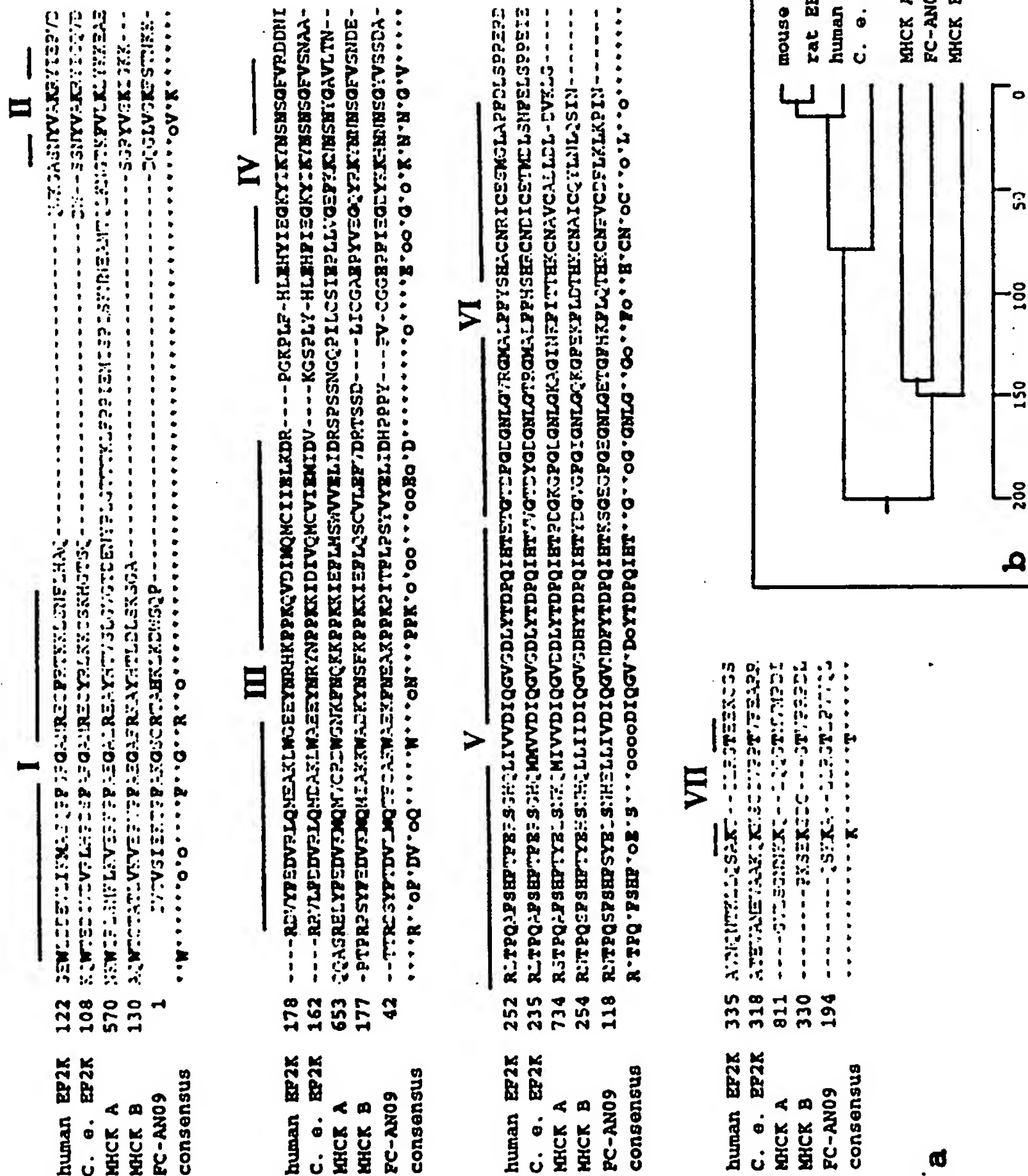


FIGURE 1

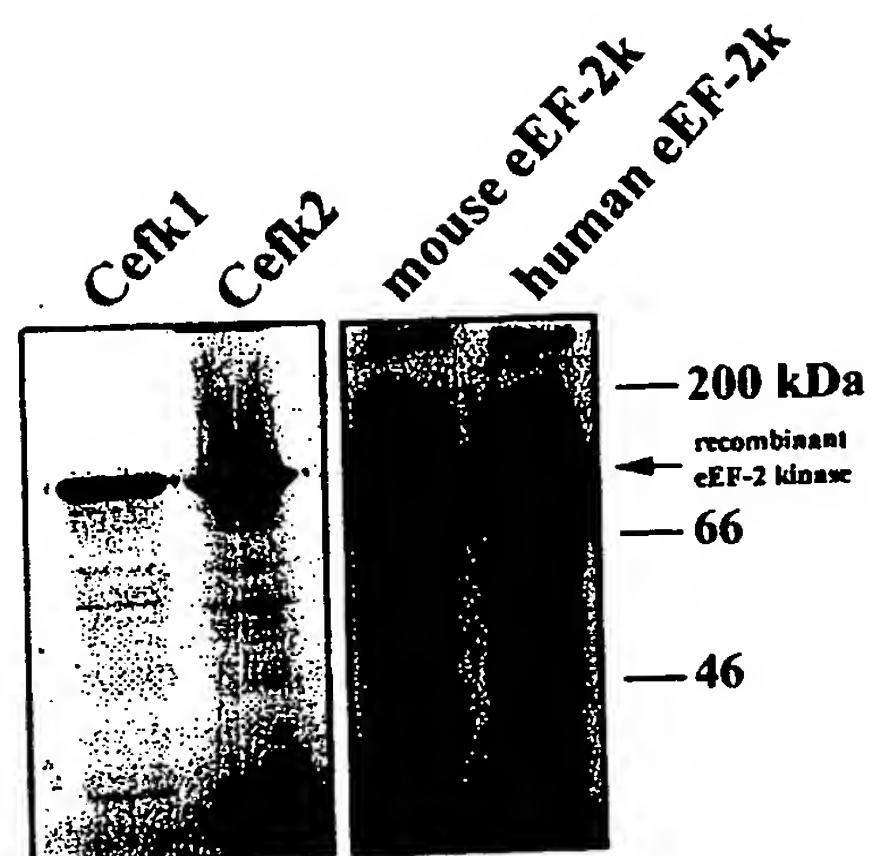


FIGURE 2

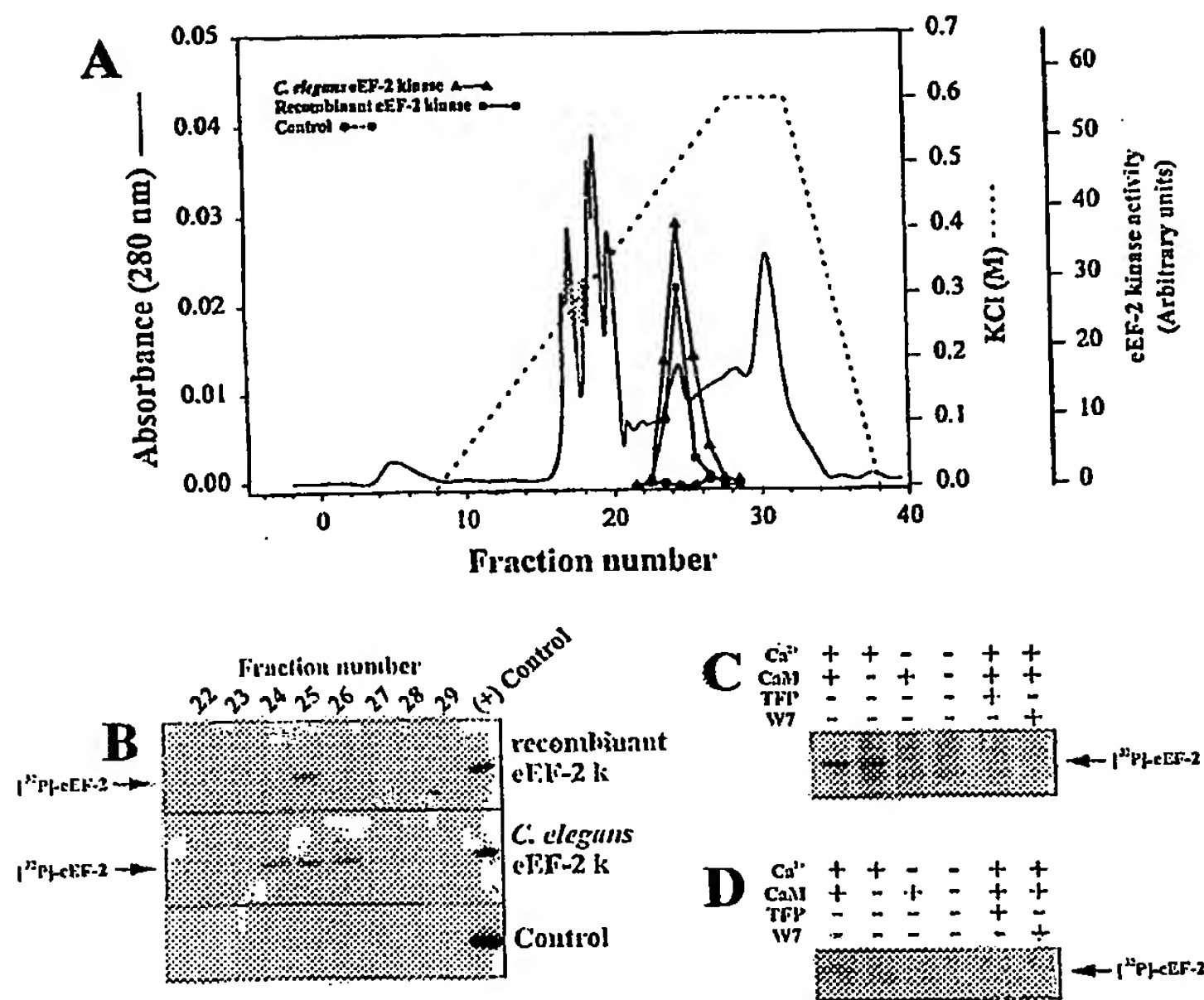


FIGURE 3

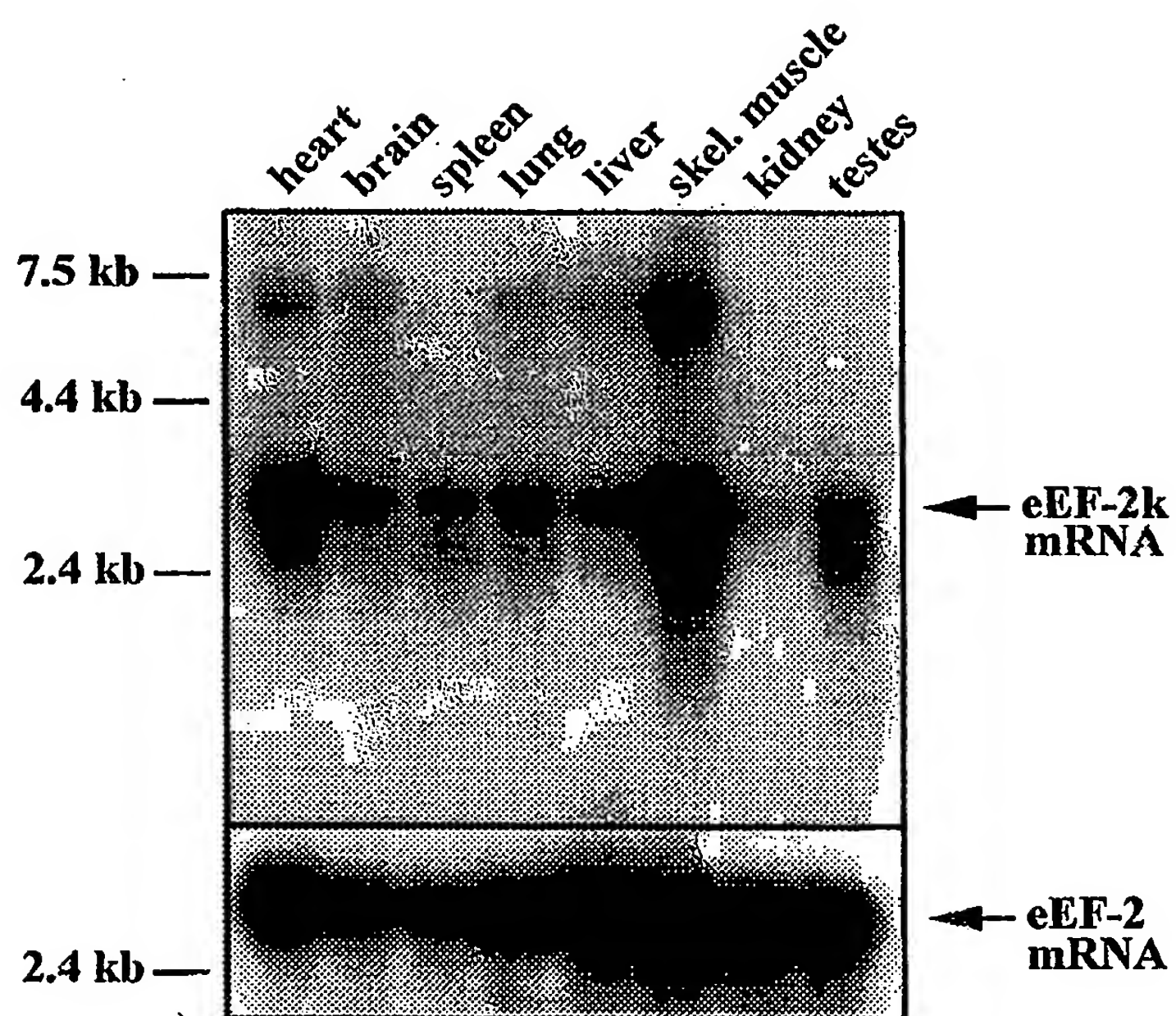


FIGURE 4

human cEF-2K	1	.....R.....QSP.....DG.....G.....B.....	44
<i>C. elegans</i> cEF-2K	1	MTIDTTNESDNGSPNGPGLSASARTFLNAKMMVR-----	39
mouse cEF-2K	1	MADEDLIFCLBGVDGGRCSRQHNADSDTDDDDDRGYFICP	44
human cEF-2K	45	..PS.....N.....NK.....S..RY..SS.....N.....Q.....	93
<i>C. elegans</i> cEF-2K	39	YADEVPISQNDVVISKPRMD-----LHVRKLMHTKARRRT	79
mouse cEF-2K	45	HMNSNQNVSXKVQBYBNLTKECGS..TGSABSVHVKELAHIS	92
human cEF-2K	94	.....R.....D.....	143
<i>C. elegans</i> cEF-2K	80	NYIDPQDEMYHSYFVQRAKYSSRKQDEIDIPDQATQ	129
mouse cEF-2K	93	HMPDPTAKKKEIATEHATHNNTQGEELASQOQDQ	142
NIHCKA	572	.....LSKK.....VERKTFAYAL	591
human cEF-2K	144	.....	166
<i>C. elegans</i> cEF-2K	130	.....KHGTSQDS--S	150
mouse cEF-2K	143	.....HPLHAQQKQAK	163
NIHCKA	592	.....VGTDSNYPLQTTTKLPPIBHIDPISKNNEAHTQLKNGK	641
human cEF-2K	167	.....D.....E.....	212
<i>C. elegans</i> cEF-2K	151	YAKKRYCQ-----RRCLFSTYQMDAPLAEKXNPPKRI	196
mouse cEF-2K	166	YAKKRYCQ-----RSYFSDYQLOHAKLGCSTNKHKPFKDT	211
NIHCKA	642	YKLYKKEANQQAERELPFSTYQMDAPLAEKXNPPKRI	691
human cEF-2K	213	.....K.....	256
<i>C. elegans</i> cEF-2K	197	YEMSAVK-----QBP-----VSEYEGRYLKMSV--IAA	239
mouse cEF-2K	213	YEMSAVK-----QBP-----VSEYEGRYLKMSV--IAA	253
NIHCKA	692	WVVRRTDRSPSBNQQPICSNPLVGEFKKXNNTYAVC--RDSQ	730
human cEF-2K	257	.....T.....	306
<i>C. elegans</i> cEF-2K	240	AEIIPYEFKHKVVDQOYELSPITAVVVDQOYELSPITAV	289
mouse cEF-2K	256	AEIIPYEFKHKVVDQOYELSPITAVVVDQOYELSPITAV	303
NIHCKA	739	AEIIPYEFKHKVVDQOYELSPITAVVVDQOYELSPITAV	788
human cEF-2K	307	.....E.....A.....R.....N..Z.....	354
<i>C. elegans</i> cEF-2K	290	CFPHSHCHDSCDNLNDEIAEATGVAMZAAKQKSCPPPV	339
mouse cEF-2K	306	CFPHSHCHDSCDNLNDEIAEATGVAMZAAKQKSCPPPV	353
NIHCKA	789	KPIITENAVCALP	805
human cEF-2K	355	.....V.....G.....RP.....	400
<i>C. elegans</i> cEF-2K	340	FEARRHSGZCVHVEHGIGSDQKRRKTL-----QSS	386
mouse cEF-2K	354	EKCGSTPRLSGG-----RPEL-----LSENGQDLHM	399
human cEF-2K	401	.....G.....	449
<i>C. elegans</i> cEF-2K	387	CFECIPVVEQCCKCSKDEFEDESDYPRSEKSONSQKRRSRNBI	436
mouse cEF-2K	400	TFHSGQKDHCHVFGDLNMGPRDHDRMDUNRDSENGQDSGYPR	448
human cEF-2K	450	.....Y..-K.....	494
<i>C. elegans</i> cEF-2K	437	GESADRRCCKFVDLNLQLRHSPFRSGYSMNSSRQFRDTEKDEFW	486
mouse cEF-2K	449	LDDEBRREH--HSGN--RHSDSD--GGRVCVEENNLNPFRL	493
human cEF-2K	495	.....A.....EK.....	532
<i>C. elegans</i> cEF-2K	487	ELRKQSVFANI LDQLQAANFENDEEDVPVUTONHQF	536
mouse cEF-2K	494	ELRKQSVFANI LDQLQAANFENDEEDVPVUTONHQF	531
human cEF-2K	533	.....Q.....V.....N.....	585
<i>C. elegans</i> cEF-2K	517	HELEFVVSSEHHEMLEGSHNDARVPITKYDQSAIFDIARKCGI	586
mouse cEF-2K	532	HELEFVVSSEHHEMLEGSHNDARVPITKYDQSAIFDIARKCGI	584
human cEF-2K	566	.....	596
<i>C. elegans</i> cEF-2K	547	YVATSAHIVLGDFHXXDLPFNGVGEQESADKQKQKPCDLBB	636
mouse cEF-2K	565	YVATSAHIVLGDFHXXDLPFNGVGEQESADKQKQKPCDLBB	595
human cEF-2K	597	.....Q.....S.....L.....	645
<i>C. elegans</i> cEF-2K	637	PQSDEIAAFHDDQALUYAHXSDQLHGLNBRDYKKS	606
mouse cEF-2K	596	PQSDEIAAFHDDQALUYAHXSDQLHGLNBRDYKKS	644
human cEF-2K	646	.....H.....N.....R..MM.....V.....Y.....E..D.....	689
<i>C. elegans</i> cEF-2K	687	OPQSEBELDDDCCKTTSBPAPLTRHSEMAEMXYKBCCQLNQDPSA	736
mouse cEF-2K	645	OPQSEBELDDDCCKTTSBPAPLTRHSEMAEMXYKBCCQLNQDPSA	689
human cEF-2K	690	.....Q.....	725
<i>C. elegans</i> cEF-2K	737	YGLNEAPAAANNNKLNKRTFAKNC--GE	760
mouse cEF-2K	689	YGLNEAPAAANNNKLNKRTFAKNC--GE	724

FIGURE 5

SUBSTITUTE SHEET (RULE 26)

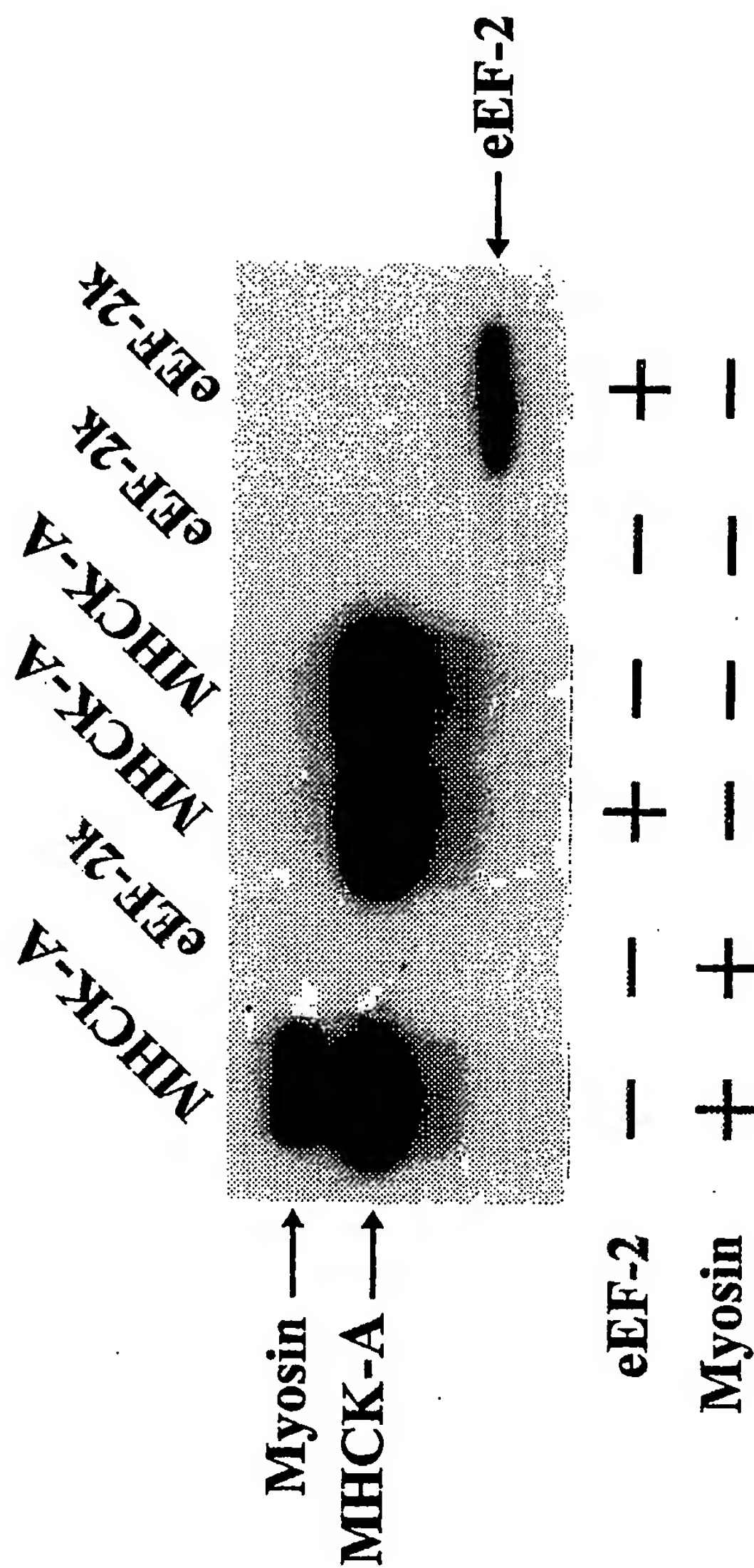


FIGURE 6

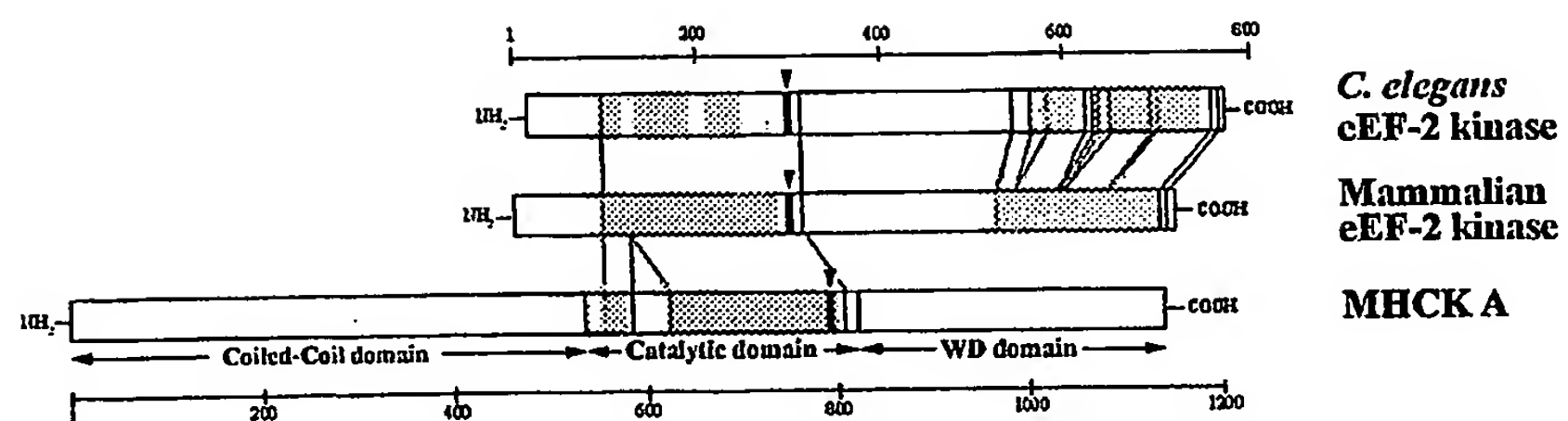


FIGURE 7



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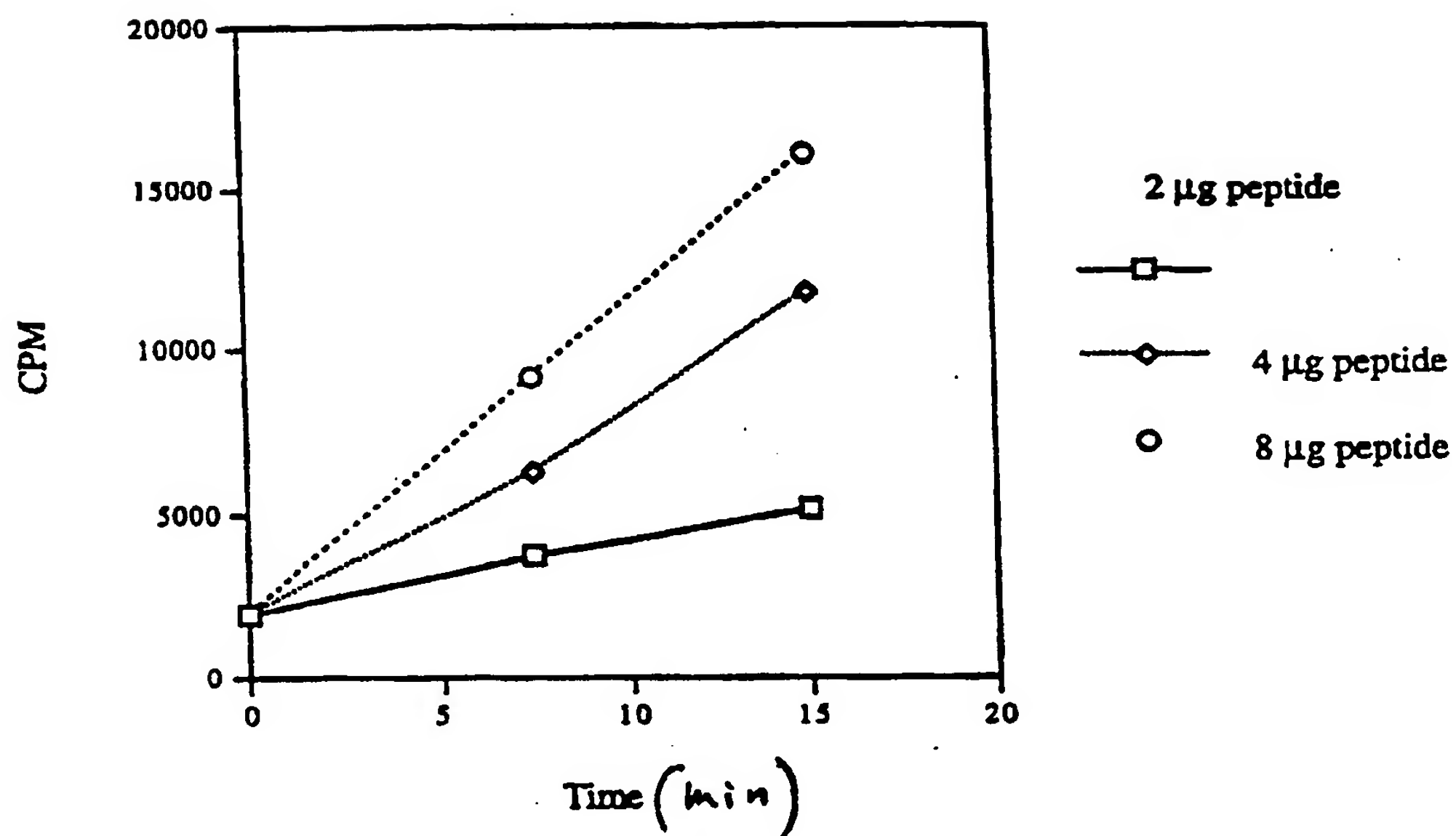


FIGURE 8

I		II	
H. EF2K	124	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
C.e. EF2K	110	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK A	572	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK B	132	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK C	48	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
heart K	189	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
melano K	48	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
ch 4 K	1127	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
consensus		GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
III		IV	
H. EF2K	178	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
C.e. EF2K	162	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK A	653	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK B	177	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK C	89	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
heart K	249	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
melano K	108	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
ch 4 K	1173	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
consensus		GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
V		VI	
H. EF2K	252	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
C.e. EF2K	235	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK A	734	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK B	254	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK C	165	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
heart K	335	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
melano K	189	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
ch 4 K	1253	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
consensus		GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
VII		VIII	
H. EF2K	252	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
C.e. EF2K	235	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK A	734	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK B	254	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
MHCK C	165	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
heart K	335	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
melano K	189	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
ch 4 K	1253	GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD
consensus		GGDDSLIMMAGIFAGGMRGFFANLSTLSTQ	GGKAGNYVARYIIEFD

FIGURE 9

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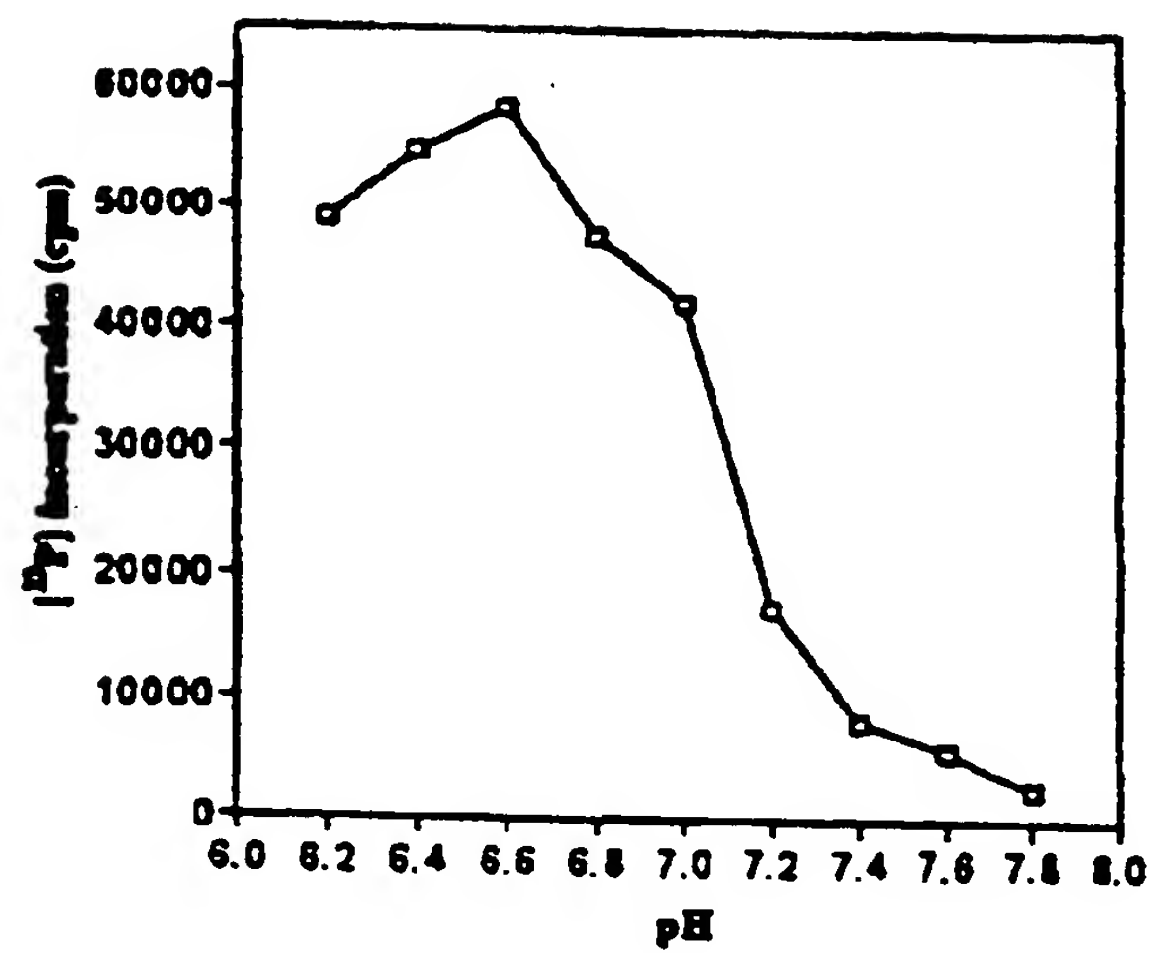


FIGURE 10

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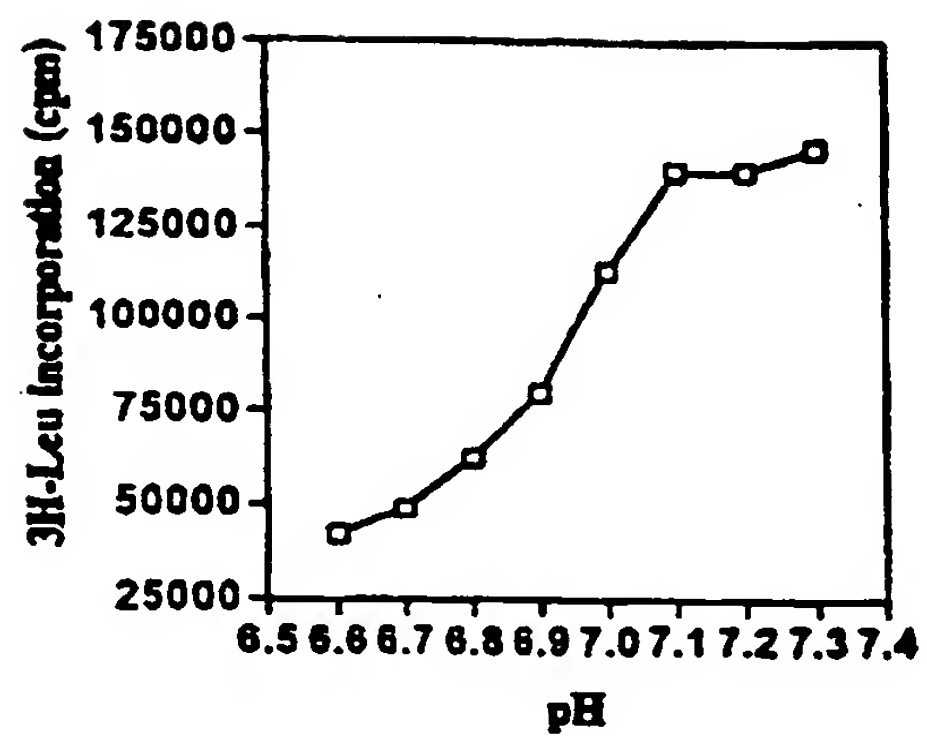


FIGURE 11

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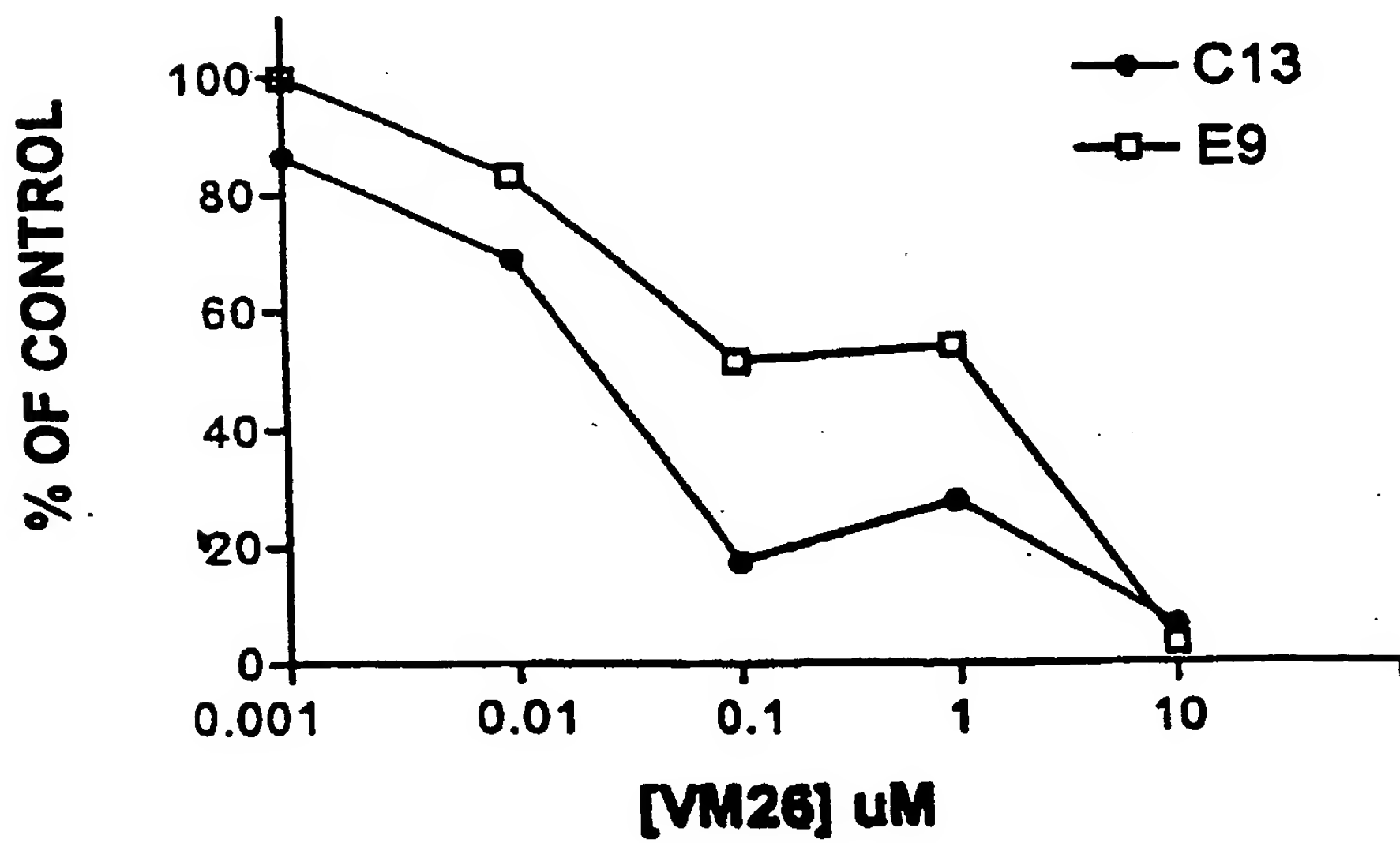
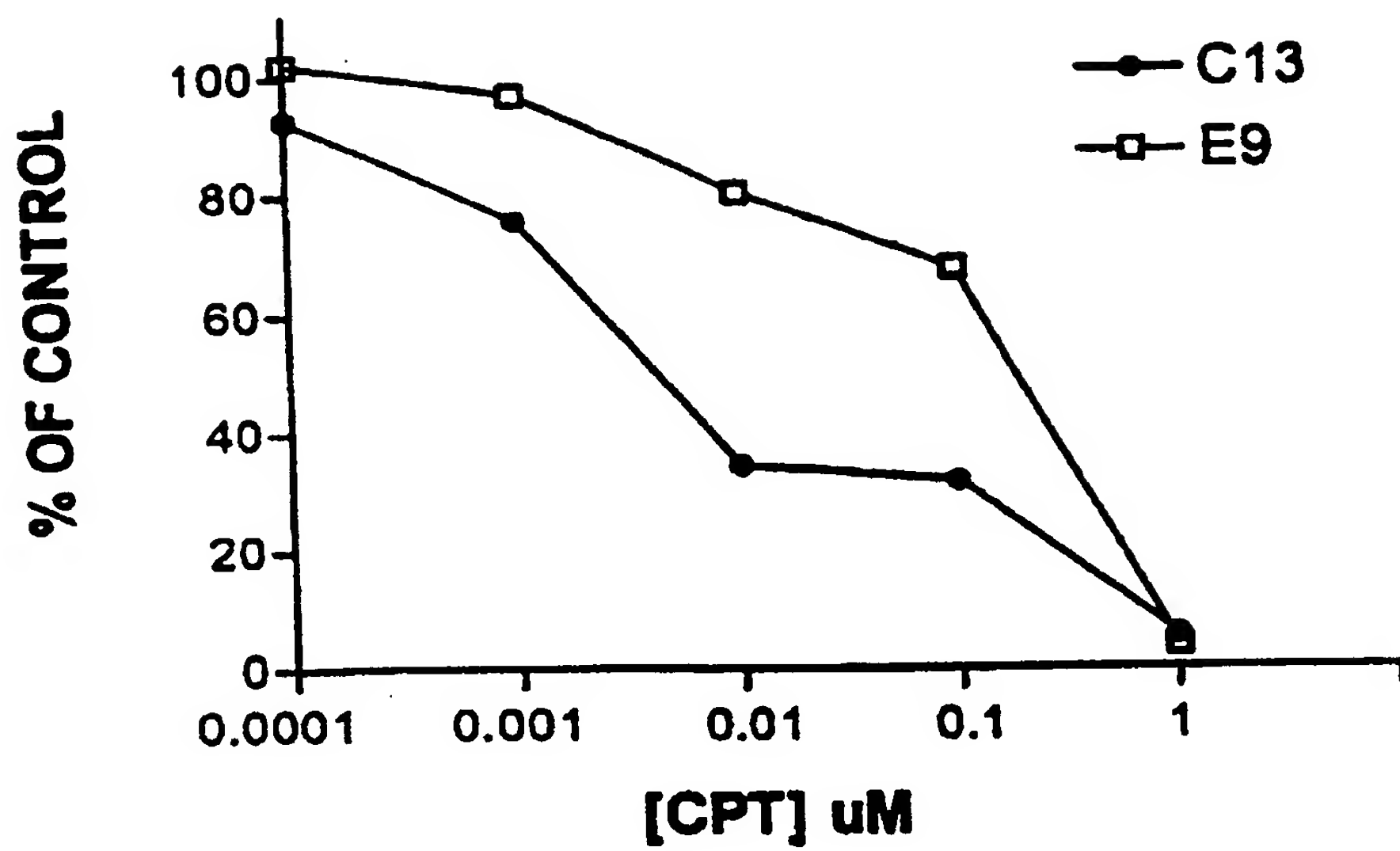


FIGURE 12

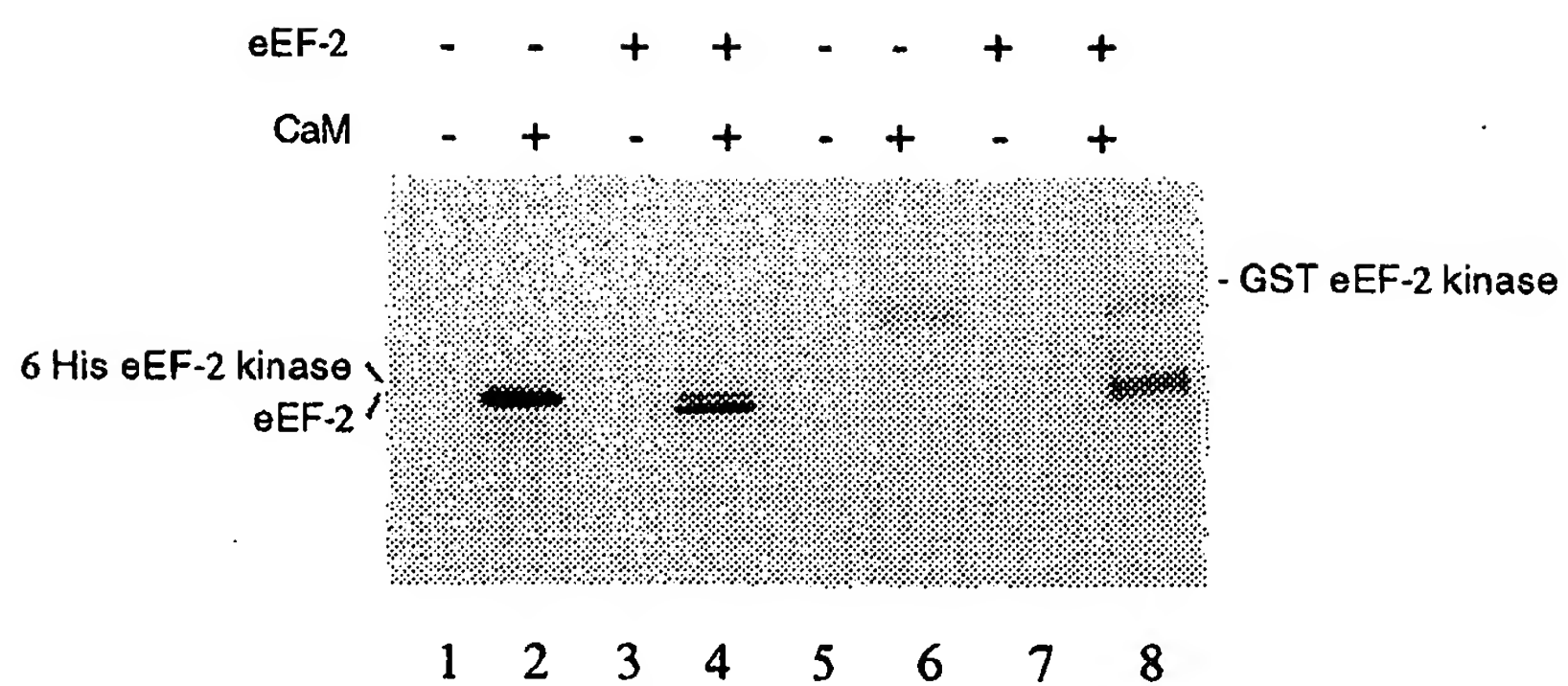


FIGURE 13

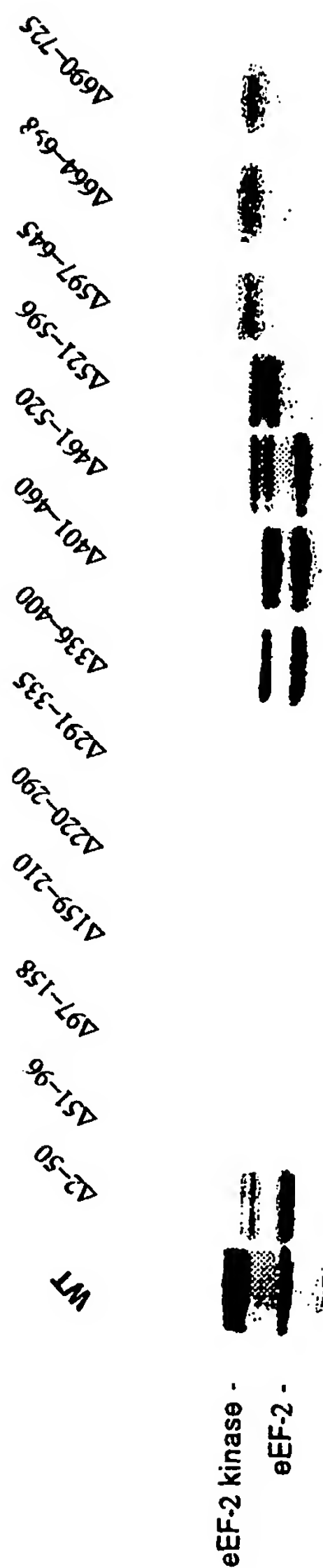


FIGURE 14



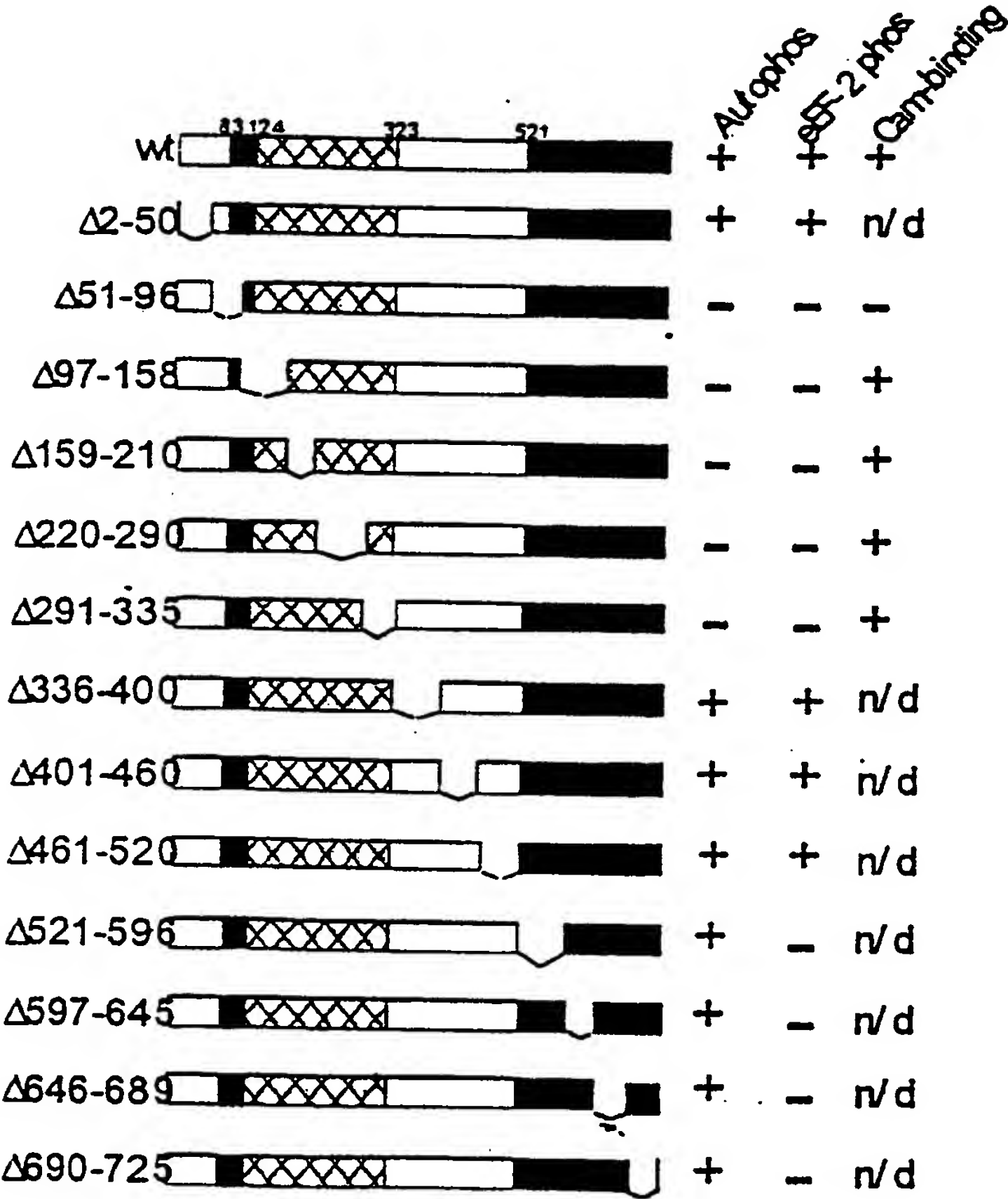
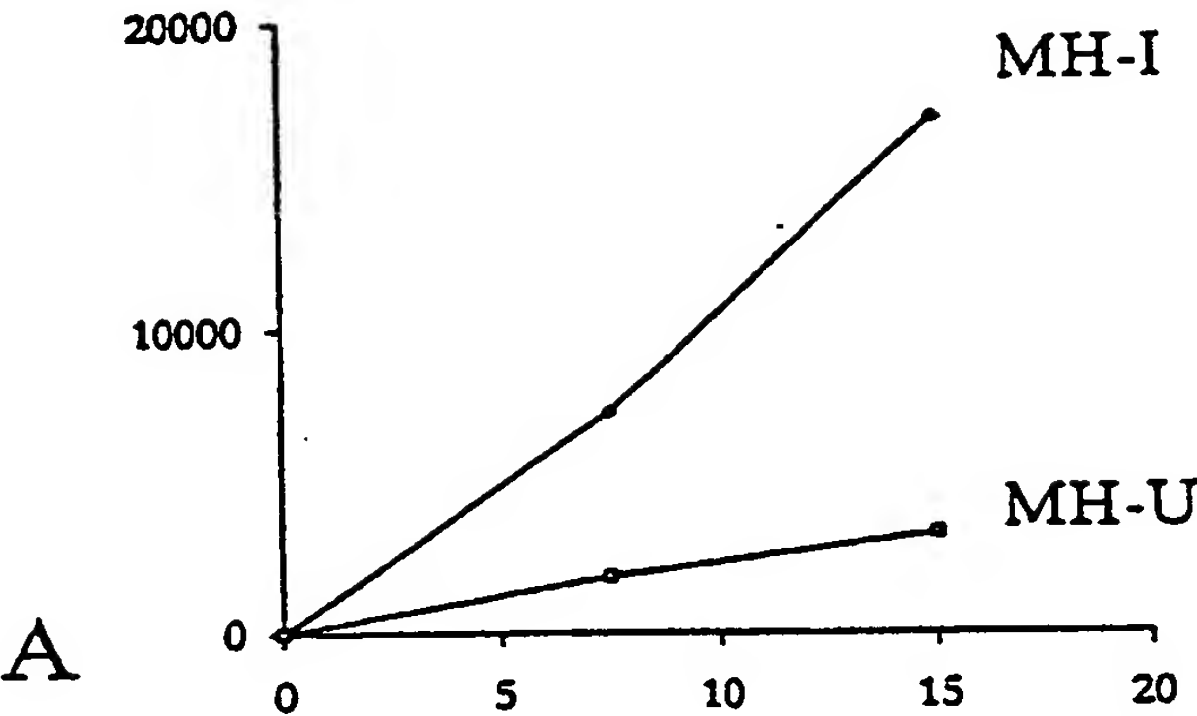


FIGURE 15



\*  
RKKFGESEKTKTKEFL - MHC  
SARAGETRFTDTRKDE - EF2

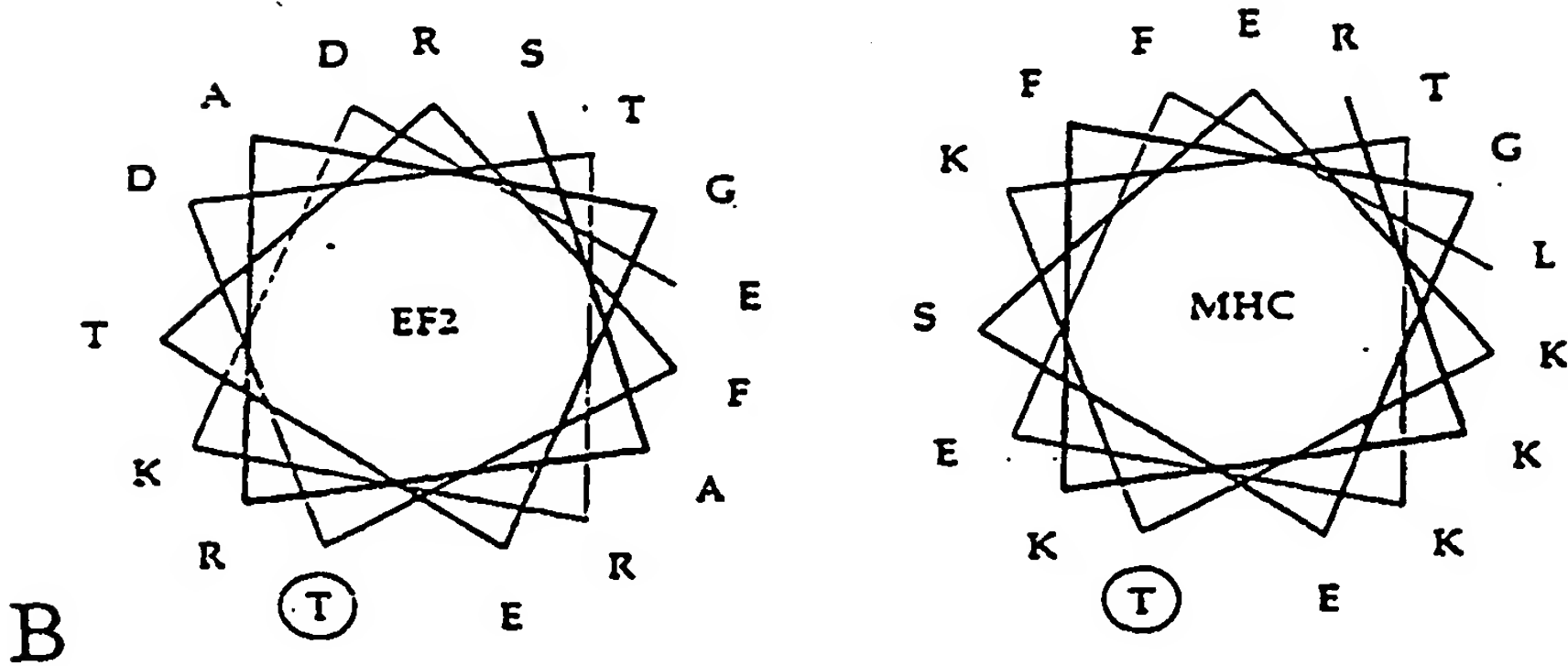


FIGURE 16

-1-

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Ryazanov, Alexey G.  
Hait, William N.  
Pavur, Karen S.
- (ii) TITLE OF INVENTION: ELONGATION FACTOR-2 KINASE (EF-2 KINASE)  
AND METHODS OF USE THEREFOR
- (iii) NUMBER OF SEQUENCES: 25
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: David A. Jackson, Esq.
  - (B) STREET: 411 Hackensack Ave, Continental Plaza, 4th  
Floor
  - (C) CITY: Hackensack
  - (D) STATE: New Jersey
  - (E) COUNTRY: USA
  - (F) ZIP: 07601
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: US
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Jackson Esq., David A.
  - (B) REGISTRATION NUMBER: 26,742
  - (C) REFERENCE/DOCKET NUMBER: 601-1-078
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 201-487-5800
  - (B) TELEFAX: 201-343-1684

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2178 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

-2-

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGCAGACG AAGACCTCAT CTTCCGCCTG GAAGGTGTTG ATGGCGGCCA GTCCCCCCGA	60
GCTGGCCATG ATGGTGATTC TGATGGGGAC AGCGACGATG AGGAAGGTTA CTTTCATCTGC	120
CCCATCACGG ATGACCCAAG CTCGAACCAG AATGTCAATT CCAAGGTTAA TAAGTACTAC	180
AGCAACCTAA CAAAAAGTGA GCGGTATAGC TCCAGCGGGT CCCC GGCAAA CTCCTTCCAC	240
TTCAAGGAAG CCTGGAAGCA CGCAATCCAG AAGGCCAAGC ACATGCCCCG CCCCTGGGCT	300
GAGTTCCACC TGGAAGATAT TGCCACCGAA CGTGCTACTC GACACAGGTA CAACGCCGTC	360
ACCGGGGAAT GGCTGGATGA TGAAGTTCTG ATCAAGATGG CATCTCAGCC CTTCCGGCCGA	420
GGAGCAATGA GGGAGTGCTT CCGGACGAAG AAGCTCTCCA ACTTCTTGCA TGCCCAGCAG	480
TGGAAGGGCG CCTCCAATA CGTGGCGAAG CGCTACATCG AGCCCGTAGA CCGGGATGTG	540
TACTTTGAGG ACGTGCGTCT ACAGATGGAG GCCAAGCTCT GGGGGGAGGA GTATAATCGG	600
CACAAGCCCC CCAAGCAGGT GGACATCATG CAGATGTGCA TCATCGAGCT GAAGGACAGA	660
CCGGGCAAGC CCCTCTTCCA CCTGGAGCAC TACATCGAGG GCAAGTACAT CAAGTACAAC	720
TCCAACCTCTG GCTTTGTCCG TGATGACAAC ATCCGACTGA CGCCGCAGGC CTTCAGCCAC	780
TTCACTTTTG AGCGTTCCGG CCATCAGCTG ATAGTGGTGG ACATCCAGGG AGTTGGGGAT	840
CTCTACACTG ACCCACAGAT CCACACGGAG ACGGGCACTG ACTTTGGAGA CGGCAACCTA	900
GGTGTCCGCG GGATGGCGCT CTTCTTCTAC TCTCATGCCT GCAACCGGAT TTGCGAGAGC	960
ATGGGCCTTG CTCCCTTTGA CCTCTCGCCC CGGGAGAGGG ATGCAGTGAA TCAGAACACC	1020
AAGCTGCTGC AATCAGCCAA GACCATCTTG AGAGGAACAG AGGAAAAATG TGGGAGCCCC	1080
CGAGTAAGGA CCCTCTCTGG GAGCCGGCCA CCCCTGCTCC GTCCCCTTTC AGAGAACTCT	1140
GGAGACGAGA ACATGAGCGA CGTGACCTTC GACTCTCTCC CTTCTTCCCC ATCTTCGGCC	1200

ACACCACACA GCCAGAAGCT AGACCACCTC CATTGGCCAG TGTTTCAGTGA CCTCGATAAC	1260
ATGGCATCCA GAGACCATGA TCATCTAGAC AACCACCGGG AGTCTGAGAA TAGTGGGGAC	1320
AGCGGATACC CCAGTGAGAA GCGGGGTGAG CTGGATGACC CTGAGCCCCG AGAACATGGC	1380
CACTCATACA GTAATCGGAA GTACGAGTCT GACGAAGACA GCCTGGGCAG CTCTGGACGG	1440
GTATGTGTAG AGAAGTGGAA TCTCCTCAAC TCCTCCCGCC TCCACCTGCC GAGGGCTTCG	1500
GCCGTGGCCC TGGAAGTGCA AAGGCTTAAT GCTCTGGACC TCGAAAAGAA AATCGGGAAG	1560
TCCATTTTGG GGAAGGTCCA TCTGGCCATG GTGCGCTACC ACGAGGGTGG GCGCTTCTGC	1620
GAGAAGGGCG AGGAGTGGGA CCAGGAGTCG GCTGTCTTCC ACCTGGAGCA CGCAGCCAAC	1680
CTGGGCGAGC TGGAGGCCAT CGTGGGCCTG GGACTCATGT ACTCGCAGTT GCCTCATCAC	1740
ATCCTAGCCG ATGTCTCTCT GAAGGAGACA GAAGAGAACA AAACCAAAGG ATTTGATTAC	1800
TTACTAAAGG CCGCTGAAGC TGGCGACAGG CAGTCCATGA TCCTAGTGGC GCGAGCTTTT	1860
GACTCTGGCC AGAACCTCAG CCCGGACAGG TGCCAAGACT GGCTAGAGGC CCTGCACTGG	1920
TACAACACTG CCCTGGAGAT GACGGACTGT GATGAGGGCG GTGAGTACGA CGGAATGCAG	1980
GACGAGCCCC GGTACATGAT GCTGGCCAGG GAGGCAGAGA TGCTGTTCAC AGGAGGCTAC	2040
GGGCTGGAGA AGGACCCGCA GAGATCAGGG GACTTGTATA CCCAGGCAGC AGAGGCAGCG	2100
ATGGAAGCCA TGAAGGGCCG ACTGGCCAAC CAGTACTACC AAAAGGCTGA AGAGGCCTGG	2160
GCCCAGATGG AGGAATAA	2178

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 725 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ala	Asp	Glu	Asp	Leu	Ile	Phe	Arg	Leu	Glu	Gly	Val	Asp	Gly	Gly	1	5	10	15
Gln	Ser	Pro	Arg	Ala	Gly	His	Asp	Gly	Asp	Ser	Asp	Gly	Asp	Ser	Asp	20	25	30	
Asp	Glu	Glu	Gly	Tyr	Phe	Ile	Cys	Pro	Ile	Thr	Asp	Asp	Pro	Ser	Ser	35	40	45	
Asn	Gln	Asn	Val	Asn	Ser	Lys	Val	Asn	Lys	Tyr	Tyr	Ser	Asn	Leu	Thr	50	55	60	
Lys	Ser	Glu	Arg	Tyr	Ser	Ser	Ser	Gly	Ser	Pro	Ala	Asn	Ser	Phe	His	65	70	75	80
Phe	Lys	Glu	Ala	Trp	Lys	His	Ala	Ile	Gln	Lys	Ala	Lys	His	Met	Pro	85	90	95	
Asp	Pro	Trp	Ala	Glu	Phe	His	Leu	Glu	Asp	Ile	Ala	Thr	Glu	Arg	Ala	100	105	110	
Thr	Arg	His	Arg	Tyr	Asn	Ala	Val	Thr	Gly	Glu	Trp	Leu	Asp	Asp	Glu	115	120	125	
Val	Leu	Ile	Lys	Met	Ala	Ser	Gln	Pro	Phe	Gly	Arg	Gly	Ala	Met	Arg	130	135	140	
Glu	Cys	Phe	Arg	Thr	Lys	Lys	Leu	Ser	Asn	Phe	Leu	His	Ala	Gln	Gln	145	150	155	160
Trp	Lys	Gly	Ala	Ser	Asn	Tyr	Val	Ala	Lys	Arg	Tyr	Ile	Glu	Pro	Val	165	170	175	
Asp	Arg	Asp	Val	Tyr	Phe	Glu	Asp	Val	Arg	Leu	Gln	Met	Glu	Ala	Lys	180	185	190	
Leu	Trp	Gly	Glu	Glu	Tyr	Asn	Arg	His	Lys	Pro	Pro	Lys	Gln	Val	Asp	195	200	205	
Ile	Met	Gln	Met	Cys	Ile	Ile	Glu	Leu	Lys	Asp	Arg	Pro	Gly	Lys	Pro	210	215	220	
Leu	Phe	His	Leu	Glu	His	Tyr	Ile	Glu	Gly	Lys	Tyr	Ile	Lys	Tyr	Asn	225	230	235	240
Ser	Asn	Ser	Gly	Phe	Val	Arg	Asp	Asp	Asn	Ile	Arg	Leu	Thr	Pro	Gln	245	250	255	
Ala	Phe	Ser	His	Phe	Thr	Phe	Glu	Arg	Ser	Gly	His	Gln	Leu	Ile	Val				

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260					265					270					
Val	Asp	Ile	Gln	Gly	Val	Gly	Asp	Leu	Tyr	Thr	Asp	Pro	Gln	Ile	His
	275						280					285			
Thr	Glu	Thr	Gly	Thr	Asp	Phe	Gly	Asp	Gly	Asn	Leu	Gly	Val	Arg	Gly
	290					295					300				
Met	Ala	Leu	Phe	Phe	Tyr	Ser	His	Ala	Cys	Asn	Arg	Ile	Cys	Glu	Ser
	305					310					315				320
Met	Gly	Leu	Ala	Pro	Phe	Asp	Leu	Ser	Pro	Arg	Glu	Arg	Asp	Ala	Val
				325					330					335	
Asn	Gln	Asn	Thr	Lys	Leu	Leu	Gln	Ser	Ala	Lys	Thr	Ile	Leu	Arg	Gly
			340					345					350		
Thr	Glu	Glu	Lys	Cys	Gly	Ser	Pro	Arg	Val	Arg	Thr	Leu	Ser	Gly	Ser
		355					360					365			
Arg	Pro	Pro	Leu	Leu	Arg	Pro	Leu	Ser	Glu	Asn	Ser	Gly	Asp	Glu	Asn
	370					375					380				
Met	Ser	Asp	Val	Thr	Phe	Asp	Ser	Leu	Pro	Ser	Ser	Pro	Ser	Ser	Ala
	385					390					395				400
Thr	Pro	His	Ser	Gln	Lys	Leu	Asp	His	Leu	His	Trp	Pro	Val	Phe	Ser
				405					410					415	
Asp	Leu	Asp	Asn	Met	Ala	Ser	Arg	Asp	His	Asp	His	Leu	Asp	Asn	His
			420					425					430		
Arg	Glu	Ser	Glu	Asn	Ser	Gly	Asp	Ser	Gly	Tyr	Pro	Ser	Glu	Lys	Arg
		435					440					445			
Gly	Glu	Leu	Asp	Asp	Pro	Glu	Pro	Arg	Glu	His	Gly	His	Ser	Tyr	Ser
	450					455					460				
Asn	Arg	Lys	Tyr	Glu	Ser	Asp	Glu	Asp	Ser	Leu	Gly	Ser	Ser	Gly	Arg
	465					470					475				480
Val	Cys	Val	Glu	Lys	Trp	Asn	Leu	Leu	Asn	Ser	Ser	Arg	Leu	His	Leu
				485					490					495	
Pro	Arg	Ala	Ser	Ala	Val	Ala	Leu	Glu	Val	Gln	Arg	Leu	Asn	Ala	Leu
			500					505					510		
Asp	Leu	Glu	Lys	Lys	Ile	Gly	Lys	Ser	Ile	Leu	Gly	Lys	Val	His	Leu
		515					520					525			
Ala	Met	Val	Arg	Tyr	His	Glu	Gly	Gly	Arg	Phe	Cys	Glu	Lys	Gly	Glu



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530		535		540
Glu Trp Asp Gln Glu Ser Ala Val Phe His Leu Glu His Ala Ala Asn				
545		550	555	560
Leu Gly Glu Leu Glu Ala Ile Val Gly Leu Gly Leu Met Tyr Ser Gln				
	565		570	575
Leu Pro His His Ile Leu Ala Asp Val Ser Leu Lys Glu Thr Glu Glu				
	580	585		590
Asn Lys Thr Lys Gly Phe Asp Tyr Leu Leu Lys Ala Ala Glu Ala Gly				
	595	600	605	
Asp Arg Gln Ser Met Ile Leu Val Ala Arg Ala Phe Asp Ser Gly Gln				
	610	615	620	
Asn Leu Ser Pro Asp Arg Cys Gln Asp Trp Leu Glu Ala Leu His Trp				
	625	630	635	640
Tyr Asn Thr Ala Leu Glu Met Thr Asp Cys Asp Glu Gly Gly Glu Tyr				
	645	650		655
Asp Gly Met Gln Asp Glu Pro Arg Tyr Met Met Leu Ala Arg Glu Ala				
	660	665		670
Glu Met Leu Phe Thr Gly Gly Tyr Gly Leu Glu Lys Asp Pro Gln Arg				
	675	680	685	
Ser Gly Asp Leu Tyr Thr Gln Ala Ala Glu Ala Ala Met Glu Ala Met				
	690	695	700	
Lys Gly Arg Leu Ala Asn Gln Tyr Tyr Gln Lys Ala Glu Glu Ala Trp				
	705	710	715	720
Ala Gln Met Glu Glu				
		725		

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2175 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

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(A) ORGANISM: *Mus musculus*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGCAGACG AAGACCTCAT CTTCTGCCTG GAAGGTGTTG ACGGTGGCAG GTGCTCCCGA	60
GCTGGCCACA ATGCGGACTC TGACACAGAC AGTGACGATG ATGAGGGCTA TTTCATCTGC	120
CCCATCACTG ATGACCACAT GTCCAATCAG AATGTCAGCT CCAAAGTCCA GAGCTACTAT	180
AGCAACCTAA CAAAAACAGA GTGCGGCTCC ACAGGGTCAC CAGCCAGCTC CTTCCACTTC	240
AAGGAAGCCT GGAAGCATGC GATCGAGAAA GCCAAGCACA TGCCTGACCC CTGGGCTGAA	300
TTCCATCTCG AGGACATCGC CACAGAACAT GCTACTCGGC ACAGGTACAA CGCTGTCACC	360
GGGGAATGGC TGAAAGACGA GGTTCCTGATC AAGATGGCGT CTCAGCCCTT CGGCCGTGGA	420
GCAATGAGGG AGTGCTTCAG GACGAAGAAA CTCTCCAAC TCTTGACACG CCAGCAATGG	480
AAGGGGGCCT CCAACTACGT GGCCAAGCGC TACATCGAGC CGGTGGACAG GAGCGTGTAC	540
TTTGAGGATG TGCAGCTCCA GATGGAGGCG AAGCTCTGGG GGGAGGATTA CAATCGGCAC	600
AAGCCCCCA AGCAGGTGGA TATCATGCAG ATGTGCATCA TTGAGCTAAA GGACAGACCA	660
GGCCAGCCCC TCTTCCACTT GGAGCACTAC ATTGAGGGCA AGTACATCAA GTACAATTCC	720
AACTCAGGCT TTGTCCGTGA TGACAACATC CGACTAACCC CACAGGCCTT CAGCCATTTC	780
ACATTTGAGC GTTCTGGTCA TCAGCTGATT GTAGTGGACA TCCAGGGTGT GGGTGACCTT	840
TATACCGACC CACAGATCCA CACTGAGAAA GGCCTGACT TTGGAGATGG TAACCTTGGT	900
GTCCGGGGAA TGGCTCTCTT CTTCTACTCT CATGCCTGCA ACCGGATTTG TCAGAGCATG	960
GGCCTTACGC CCTTTGACCT CTCCCCACGG GAACAGGATG CGGTGAATCA GAGCACCAGG	1020
CTATTGCAAT CAGCCAAGAC CATCTTGAGG GGGACAGAGG AGAAGTGTGG GAGTCCCCGC	1080
ATAAGGACAC TCTCTAGCAG CCGGCCCCCT TTGCTCCTTC GCCTGTCAGA GAACTCCGGG	1140
GATGAGAACA TGAGTGACGT GACCTTTGAC TCTCTGCCTT CCTCCCCGTC TTCAGCTACA	1200
CCACACAGCC AGAAACTGGA CCACCTCCAT TGGCCAGTGT TTGGTGACCT CGATAACATG	1260
GGCCCTAGAG ACCATGACCG TATGGACAAT CACCGGGACT CTGAGAATAG TGGGGACAGT	1320
GGGTATCCAA GCGAGAAGCG AAGTGACCTG GATGATCCTG AGCCCCGAGA ACACGGCCAC	1380

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TCCAACGGCA ACCGAAGGCA TGAATCTGAC GAGGATAGCC TGGGCAGCTC TGGACGGGTC	1440
TGTGTGGAGA CGTGGAACCT GCTCAATCCC TCCCGCCTGC ACCTGCCGAG GCCCTCGGCC	1500
GTGGCCCTAG AAGTGCAGAG GCTAAATGCC CTGGACCTTG GAAGGAAAAT CGGGAAGTCT	1560
GTTTTGGGGA AAGTCCATTT GGCCATGGTG CGATACCACG AGGGCGGGCG CTTCTGCGAG	1620
AAGGATGAGG AGTGGGATCG AGAGTCAGCC ATCTTCCATC TGGAGCATGC AGCTGACCTG	1680
GGAGAACTGG AGGCCATCGT GGGCCTAGGC CTCATGTACT CTCAGCTGCC CCACCACATC	1740
CTGGCTGATG TCTCTCTGAA GGAGACAGAG GAGAACAAGA CAAAAGGCTT TGATTACTTA	1800
CTGAAGGCGG CAGAAGCTGG TGACAGGCAT TCCATGATTT TAGTGGCCCG AGCTTTTGAC	1860
ACTGGCCTGA ACCTCAGCCC AGACAGGTGT CAAGACTGGT CGGAAGCCTT GCACTGGTAC	1920
AACACAGCCC TGGAGACAAC AGACTGCGAT GAAGGCGGGG AGTACGATGG GATACAGGAC	1980
GAGCCCCAGT ACGCACTGCT GGCCAGGGAG GCGGAGATGC TGCTCACCGG GGGATTTGGA	2040
CTGGACAAGA ACCCCCAAAG ATCAGGAGAT TTGTACACCC AGGCAGCTGA GGCAGCAATG	2100
GAAGCCATGA AGGGCCGGCT AGCCAACCAG TACTACGAGA AGGCGGAAGA GGCCTGGGCC	2160
CAGATGGAGG AATAA	2175

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 724 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mus musculus

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Asp	Glu	Asp	Leu	Ile	Phe	Cys	Leu	Glu	Gly	Val	Asp	Gly	Gly
1				5				10						15	

Arg	Cys	Ser	Arg	Ala	Gly	His	Asn	Ala	Asp	Ser	Asp	Thr	Asp	Ser	Asp
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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20					25					30					
Asp	Asp	Glu	Gly	Tyr	Phe	Ile	Cys	Pro	Ile	Thr	Asp	Asp	His	Met	Ser
		35					40					45			
Asn	Gln	Asn	Val	Ser	Ser	Lys	Val	Gln	Ser	Tyr	Tyr	Ser	Asn	Leu	Thr
	50					55					60				
Lys	Thr	Glu	Cys	Gly	Ser	Thr	Gly	Ser	Pro	Ala	Ser	Ser	Phe	His	Phe
	65					70					75				80
Lys	Glu	Ala	Trp	Lys	His	Ala	Ile	Glu	Lys	Ala	Lys	His	Met	Pro	Asp
				85					90					95	
Pro	Trp	Ala	Glu	Phe	His	Leu	Glu	Asp	Ile	Ala	Thr	Glu	His	Ala	Thr
			100					105					110		
Arg	His	Arg	Tyr	Asn	Ala	Val	Thr	Gly	Glu	Trp	Leu	Lys	Asp	Glu	Val
		115					120					125			
Leu	Ile	Lys	Met	Ala	Ser	Gln	Pro	Phe	Gly	Arg	Gly	Ala	Met	Arg	Glu
	130					135					140				
Cys	Phe	Arg	Thr	Lys	Lys	Leu	Ser	Asn	Phe	Leu	His	Ala	Gln	Gln	Trp
	145					150					155				160
Lys	Gly	Ala	Ser	Asn	Tyr	Val	Ala	Lys	Arg	Tyr	Ile	Glu	Pro	Val	Asp
				165					170					175	
Arg	Ser	Val	Tyr	Phe	Glu	Asp	Val	Gln	Leu	Gln	Met	Glu	Ala	Lys	Leu
			180					185					190		
Trp	Gly	Glu	Asp	Tyr	Asn	Arg	His	Lys	Pro	Pro	Lys	Gln	Val	Asp	Ile
		195					200					205			
Met	Gln	Met	Cys	Ile	Ile	Glu	Leu	Lys	Asp	Arg	Pro	Gly	Gln	Pro	Leu
	210					215					220				
Phe	His	Leu	Glu	His	Tyr	Ile	Glu	Gly	Lys	Tyr	Ile	Lys	Tyr	Asn	Ser
	225					230					235				240
Asn	Ser	Gly	Phe	Val	Arg	Asp	Asp	Asn	Ile	Arg	Leu	Thr	Pro	Gln	Ala
				245					250					255	
Phe	Ser	His	Phe	Thr	Phe	Glu	Arg	Ser	Gly	His	Gln	Leu	Ile	Val	Val
			260					265					270		
Asp	Ile	Gln	Gly	Val	Gly	Asp	Leu	Tyr	Thr	Asp	Pro	Gln	Ile	His	Thr
		275					280					285			
Glu	Lys	Gly	Thr	Asp	Phe	Gly	Asp	Gly	Asn	Leu	Gly	Val	Arg	Gly	Met

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290	295	300
Ala Leu Phe Phe Tyr Ser His Ala Cys Asn Arg Ile Cys Gln Ser Met 305	310	315 320
Gly Leu Thr Pro Phe Asp Leu Ser Pro Arg Glu Gln Asp Ala Val Asn 325	330	335
Gln Ser Thr Arg Leu Leu Gln Ser Ala Lys Thr Ile Leu Arg Gly Thr 340	345	350
Glu Glu Lys Cys Gly Ser Pro Arg Ile Arg Thr Leu Ser Ser Ser Arg 355	360	365
Pro Pro Leu Leu Leu Arg Leu Ser Glu Asn Ser Gly Asp Glu Asn Met 370	375	380
Ser Asp Val Thr Phe Asp Ser Leu Pro Ser Ser Pro Ser Ser Ala Thr 385	390	395 400
Pro His Ser Gln Lys Leu Asp His Leu His Trp Pro Val Phe Gly Asp 405	410	415
Leu Asp Asn Met Gly Pro Arg Asp His Asp Arg Met Asp Asn His Arg 420	425	430
Asp Ser Glu Asn Ser Gly Asp Ser Gly Tyr Pro Ser Glu Lys Arg Ser 435	440	445
Asp Leu Asp Asp Pro Glu Pro Arg Glu His Gly His Ser Asn Gly Asn 450	455	460
Arg Arg His Glu Ser Asp Glu Asp Ser Leu Gly Ser Ser Gly Arg Val 465	470	475 480
Cys Val Glu Thr Trp Asn Leu Leu Asn Pro Ser Arg Leu His Leu Pro 485	490	495
Arg Pro Ser Ala Val Ala Leu Glu Val Gln Arg Leu Asn Ala Leu Asp 500	505	510
Leu Gly Arg Lys Ile Gly Lys Ser Val Leu Gly Lys Val His Leu Ala 515	520	525
Met Val Arg Tyr His Glu Gly Gly Arg Phe Cys Glu Lys Asp Glu Glu 530	535	540
Trp Asp Arg Glu Ser Ala Ile Phe His Leu Glu His Ala Ala Asp Leu 545	550	555 560
Gly Glu Leu Glu Ala Ile Val Gly Leu Gly Leu Met Tyr Ser Gln Leu		

565

570

575

Gln Met Glu Glu

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGTTTAATA TAAAAAAGAG AAAAGAGAGT ATAACAGGTA TACCACCAAT AAATGTTAAT	60
AGTCCACAAT CAGTTCCATT GAGTGAACA TTGCAATCAC CATTGATTAC ACCAAATTCA	120
CCAAATTTTG TTTCACGTCA ATGTCCATTC AAAAAGTTTG GATGTAGTAG TTTTTTAGTT	180
TCAAAGGCAG AGTTTGATAA TCACTTAAAG GATGACGCAC AATTTCAATT ACAATTGGCA	240
GTGGAGAAAT TTGATCATCA ATTTGATTTA CACACACAAT TGATGGCACA TTTTACTGAG	300
CAAATGGAGG ATCAATTAGA GAAAACAATG AAGGTCGTAC GTAATCATA AGATAGTTTA	360
GGCGGTAATG TTCAAACCAA ATTGGATGAA GGCATTGAAA AATGTATGGC TTTTGCTAAA	420
AAGGTTGAAC AACAACAACA ACAATTGGCC AAAAGATTAA TCACTCAACA AATTCAAGAG	480
AAGAAATCAA CCTCTTCACC TTTAGTTAAA GGTGGTATTA GTGGTGGTGG TGGTAGTGGT	540
GGCGATGATT CTTTTGATGG CGCAAATATA TCATCAATGT CAACTAGTAA ACAAGAATTA	600
CAACAAGAAT TACAATCATT ATCAATTAAA ATGAAAAAAG AATTGACAGA ATTATCCGAT	660
GAACTATCAC AAAAATTAGA ACGTTCAACA GGTAATATAG ATATTAAAAT AAAGAGAATC	720
GAAGGTGAAG TTAATGAAAA GATTGATAAA CGTCAATTGG TCTCTACGAT CGATGATTCA	780
ATTGGAAAGA AAACAGATTC CATCGGTTAT ACATTGGAGA GTTCAATCAT TAAAAAGGTT	840
GAAGAGAAAG AGAAAAAGAA ATCCGAACAA AATCAACTTC TCTTTGATTC AAAGATTGAA	900
TCCTTAAAAG ATAAGATTAA AATCATTGAA ACTCAACAAT TGGATACTTC ATCAGAGGTT	960
AGAAAATTGA AATTAGAAAG TACAAGTAGT GGAAATTTAA TGGCAGGTCT TAATGGTACC	1020
TCTGGTAGAC CTTCATCATC TTCTCACTTT ATTCCATCCT CTGTTTCTGC CGCTGCTAAC	1080
AATATCAACA AGAATGAAAT CATGGAAGAG GTTAAAAAGG TAGAAGAGAA ACTTCAAAAG	1140
AAAATTCGTG AAGAGATTGA TAATACAAAA GCTGAACTCT CAAAGGTGTA ACGTTCCGTT	1200
AAAGATAATC GTAGTGAAAT TGAAGGTTTG GAAAAAGATT GTAAGAATCA ATTCGATAAA	1260
CAAGACAATA AGATCAAACA AGTTGAGGAT GATTTGAAAA AGAGTGATTC ATTACTTTTG	1320
TTAATGCAAA ATAACCTCAA GAAATATAAT GAATTTGTTG ATAGAGAACG TGATCGTGAA	1380
AGTGAACGTT TGAAACTTCA AGATTCTATC AAACGTTTAG AACAAAATCA AAAGAAAATC	1440
GAAGCTGAAA TTCAAGAAGG TAATGAACAA GTTGAACGTG TTTTACGTGA GGAAGCTTCA	1500
ATCTCACCAA TTAGTTCAGT TCCAAAATCA CCAATCACAA CCAAACGTTC ATCGATTATT	1560



TTAAATTCAC CACCAATGAC TTCACAACAA TCATCACCAA AGATTCAAGA TCTTCTCTCA	1620
AGTAGTGGTA GTAGTAGTGT TAGTGGTATA AATATTTCCCT CTGAAACCGG TGAAATGGGT	1680
ATTCTTTGGG AATTTGATCC AATCATTAAC AAATGGATTA GATTATCAAT GAAGCTAAAG	1740
GTAGAAAGAA AACCATTTGC AGAGGGTGCT CTTAGAGAGG CTTATCATAC CGTTTCATTG	1800
GGTGTGGAA CCGATGAAAA TTATCCATTA GGTACAACCA CCAAATTATT CCCACCAATT	1860
GAAATGATTT CACCAATTTT AAAGAATAAT GAGGCAATGA CTCAATTGAA GAATGGTACA	1920
AAATTTGTTT TGAAACTCTA CAAAAGGAA GCTGAACAAC AAGCTAGCAG AGAATTATAC	1980
TTTGAAGATG TTAAAATGCA AATGGTCTGT AGAGATTGGG GTAATAAATT CAATCAAAAG	2040
AAACCACCAA AGAAAATTGA ATTCCTTATG TCTTGGGTTG TAGAGTTAAT CGATAGATCT	2100
CCTTCTTCCA ATGGTCAACC AATACTTTGT TCCATTGAAC CATTATTGGT TGGTGAATTC	2160
AAAAAGAATA ATTCAAATTA TGGTGCAGTT TTAACCAATC GTTCAACTCC ACAAGCATTC	2220
TCTCATTTCA CCTATGAACT CTCAAATAAA CAAATGATCG TTGTCGATAT TCAAGGTGTT	2280
GATGATCTTT ACACTGATCC TCAAATTCAT ACACCCGATG GTAAAGGATT TGGTCTTGGT	2340
AATCTTGGTA AAGCAGGTAT CAATAAATTC ATCACCCTC ACAAATGTAA TGCTGTTTGT	2400
GCTCTTTTAG ATTTAGATGT TAAATTGGGT GGTGTACTAT CTGGAAATAA TAAGAAACAA	2460
CTTCAACAAG GTACTATGGT TATGCCAGAT ATTCTCCCAG AACTTATGCC ATCTGATAAC	2520
ACCATTAAAG TGGGTGCAAA ACAACTTCCA AAAGCTGAAT TCTCAAAGAA AGATCTCAA	2580
TGTGTTAGCA CCATTCAAAG TTTCCGTGAA CGTGTTAACT CGATCGCATT CTTTGATAAT	2640
CAAAAGTTAT TATGCGCTGG TTATGGTGAT GGTACCTATA GAGTTTTCGA TGTCAATGAC	2700
AATTGGAAAT GTTTATACAC TGTCAATGGT CATAGAAAAT CAATTGAAAG TATCGCTTGT	2760
AATAGTAATT ACATTTTCAC TTCATCACCT GATAACACCA TCAAAGTTCA TATCATTCGT	2820
AGTGGAACA CCAAATGTAT AGAGACATTG GTTGGTCACA CTGGTGAAGT TAATTGTGTC	2880
GTGGCCAATG AAAAATATCT TTTCAGTTGT AGTTATGATA AACTATCAA GGTTTGGGAT	2940
TTGTCAACCT TTAAAGAAAT TAAATCATTT GAGGGTGTTT ATACAAAGTA CATTAAAACA	3000
TTGGCTTTGA GTGGACGTTA TCTTTTGTAGT GGTGGTAACG ATCAAATCAT TTACGTTTGG	3060
GATACTGAAA CACTTAGTAT GCTTTTCAAT ATGCAAGGTC ATGAAGATTG GGTACTCTCT	3120

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CTTCATTGTA CCGCTAGTTA TCTTTTCTCA ACCTCAAAAG ATAATGTCAT CAAGATTG 3180  
 GATCTCTCAA ATTTCAAGTTG TATCGATACT CTAAAAGGTC ATTGGAATTC TGTCTCAAGT 3240  
 TGTGTCGTAA AAGATCGTTA TCTATACAGT GGTTCTGAAG ATAATTCAAT CAAAGTTTGG 3300  
 GATCTCGATA CACTTGAATG TGTTTACACC ATTCCAAAAT CTCATTCTTT GGGTGTAATA 3360  
 TGTTTAATGG TTTTCAATAA TCAAATCATT TCTGCTGCTT TCGATGGTTC AATTAAAGTT 3420  
 TGGGAATGGC AATCGAAATA ATCTTTGTAA ATTTTGTGTA AAAAA 3465

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1146 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Dictyostelium discoideum

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Phe	Asn	Ile	Lys	Lys	Arg	Lys	Glu	Ser	Ile	Thr	Gly	Ile	Pro	Pro	1	5	10	15
Ile	Asn	Val	Asn	Ser	Pro	Gln	Ser	Val	Pro	Leu	Ser	Gly	Thr	Leu	Gln	20	25	30	
Ser	Pro	Leu	Ile	Thr	Pro	Asn	Ser	Pro	Asn	Phe	Val	Ser	Arg	Gln	Cys	35	40	45	
Pro	Phe	Lys	Lys	Phe	Gly	Cys	Ser	Ser	Phe	Leu	Val	Ser	Lys	Ala	Glu	50	55	60	
Phe	Asp	Asn	His	Leu	Lys	Asp	Asp	Ala	Gln	Phe	His	Leu	Gln	Leu	Ala	65	70	75	80
Val	Glu	Lys	Phe	Asp	His	Gln	Phe	Asp	Leu	His	Thr	Gln	Leu	Met	Ala	85	90	95	
His	Phe	Thr	Glu	Gln	Met	Glu	Asp	Gln	Leu	Glu	Lys	Thr	Met	Lys	Val	100	105	110	

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Val	Arg	Asn	His	Thr	Asp	Ser	Leu	Gly	Gly	Asn	Val	Gln	Thr	Lys	Leu	115	120	125	
Asp	Glu	Gly	Ile	Glu	Lys	Cys	Met	Ala	Phe	Ala	Lys	Lys	Val	Glu	Gln	130	135	140	
Gln	Gln	Gln	Gln	Leu	Ala	Lys	Arg	Leu	Ile	Thr	Gln	Gln	Ile	Gln	Glu	145	150	155	160
Lys	Lys	Ser	Thr	Ser	Ser	Pro	Leu	Val	Lys	Gly	Gly	Ile	Ser	Gly	Gly	165	170	175	
Gly	Gly	Ser	Gly	Gly	Asp	Asp	Ser	Phe	Asp	Gly	Ala	Asn	Ile	Ser	Ser	180	185	190	
Met	Ser	Thr	Ser	Lys	Gln	Glu	Leu	Gln	Gln	Glu	Leu	Gln	Ser	Leu	Ser	195	200	205	
Ile	Lys	Met	Lys	Lys	Glu	Leu	Thr	Glu	Leu	Ser	Asp	Glu	Leu	Ser	Gln	210	215	220	
Lys	Leu	Glu	Arg	Ser	Thr	Gly	Asn	Ile	Asp	Ile	Lys	Ile	Lys	Arg	Ile	225	230	235	240
Glu	Gly	Glu	Val	Asn	Glu	Lys	Ile	Asp	Lys	Arg	Gln	Leu	Val	Ser	Thr	245	250	255	
Ile	Asp	Asp	Ser	Ile	Gly	Lys	Lys	Thr	Asp	Ser	Ile	Gly	Tyr	Thr	Leu	260	265	270	
Glu	Ser	Ser	Ile	Ile	Lys	Lys	Val	Glu	Glu	Lys	Glu	Lys	Lys	Lys	Ser	275	280	285	
Glu	Gln	Asn	Gln	Leu	Leu	Phe	Asp	Ser	Lys	Ile	Glu	Ser	Leu	Lys	Asp	290	295	300	
Lys	Ile	Lys	Ile	Ile	Glu	Thr	Gln	Gln	Leu	Asp	Thr	Ser	Ser	Glu	Val	305	310	315	320
Arg	Lys	Leu	Lys	Leu	Glu	Ser	Thr	Ser	Ser	Gly	Asn	Leu	Met	Ala	Gly	325	330	335	
Leu	Asn	Gly	Thr	Ser	Gly	Arg	Pro	Ser	Ser	Ser	Ser	His	Phe	Ile	Pro	340	345	350	
Ser	Ser	Val	Ser	Ala	Ala	Ala	Asn	Asn	Ile	Asn	Lys	Asn	Glu	Ile	Met	355	360	365	
Glu	Glu	Val	Lys	Lys	Val	Glu	Glu	Lys	Leu	Gln	Lys	Lys	Ile	Arg	Glu	370	375	380	

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Glu	Ile	Asp	Asn	Thr	Lys	Ala	Glu	Leu	Ser	Lys	Val	Glu	Arg	Ser	Val	385	390	395	400
Lys	Asp	Asn	Arg	Ser	Glu	Ile	Glu	Gly	Leu	Glu	Lys	Asp	Cys	Lys	Asn	405	410	415	
Gln	Phe	Asp	Lys	Gln	Asp	Asn	Lys	Ile	Lys	Gln	Val	Glu	Asp	Asp	Leu	420	425	430	
Lys	Lys	Ser	Asp	Ser	Leu	Leu	Leu	Leu	Met	Gln	Asn	Asn	Leu	Lys	Lys	435	440	445	
Tyr	Asn	Glu	Phe	Val	Asp	Arg	Glu	Arg	Asp	Arg	Glu	Ser	Glu	Arg	Leu	450	455	460	
Lys	Leu	Gln	Asp	Ser	Ile	Lys	Arg	Leu	Glu	Gln	Asn	Gln	Lys	Lys	Ile	465	470	475	480
Glu	Ala	Glu	Ile	Gln	Glu	Gly	Asn	Glu	Gln	Val	Glu	Arg	Val	Leu	Arg	485	490	495	
Glu	Glu	Ala	Ser	Ile	Ser	Pro	Ile	Ser	Ser	Val	Pro	Lys	Ser	Pro	Ile	500	505	510	
Thr	Thr	Lys	Arg	Ser	Ser	Ile	Ile	Leu	Asn	Ser	Pro	Pro	Met	Thr	Ser	515	520	525	
Gln	Gln	Ser	Ser	Pro	Lys	Ile	Gln	Asp	Leu	Leu	Ser	Ser	Ser	Gly	Ser	530	535	540	
Ser	Ser	Val	Ser	Gly	Ile	Asn	Ile	Ser	Ser	Glu	Thr	Gly	Glu	Met	Gly	545	550	555	560
Ile	Leu	Trp	Glu	Phe	Asp	Pro	Ile	Ile	Asn	Lys	Trp	Ile	Arg	Leu	Ser	565	570	575	
Met	Lys	Leu	Lys	Val	Glu	Arg	Lys	Pro	Phe	Ala	Glu	Gly	Ala	Leu	Arg	580	585	590	
Glu	Ala	Tyr	His	Thr	Val	Ser	Leu	Gly	Val	Gly	Thr	Asp	Glu	Asn	Tyr	595	600	605	
Pro	Leu	Gly	Thr	Thr	Thr	Lys	Leu	Phe	Pro	Pro	Ile	Glu	Met	Ile	Ser	610	615	620	
Pro	Ile	Ser	Lys	Asn	Asn	Glu	Ala	Met	Thr	Gln	Leu	Lys	Asn	Gly	Thr	625	630	635	640
Lys	Phe	Val	Leu	Lys	Leu	Tyr	Lys	Lys	Glu	Ala	Glu	Gln	Gln	Ala	Ser	645	650	655	

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Arg Glu Leu Tyr Phe Glu Asp Val Lys Met Gln Met Val Cys Arg Asp  
 660 665 670

Trp Gly Asn Lys Phe Asn Gln Lys Lys Pro Pro Lys Lys Ile Glu Phe  
 675 680 685

Leu Met Ser Trp Val Val Glu Leu Ile Asp Arg Ser Pro Ser Ser Asn  
 690 695 700

Gly Gln Pro Ile Leu Cys Ser Ile Glu Pro Leu Leu Val Gly Glu Phe  
 705 710 715 720

Lys Lys Asn Asn Ser Asn Tyr Gly Ala Val Leu Thr Asn Arg Ser Thr  
 725 730 735

Pro Gln Ala Phe Ser His Phe Thr Tyr Glu Leu Ser Asn Lys Gln Met  
 740 745 750

Ile Val Val Asp Ile Gln Gly Val Asp Asp Leu Tyr Thr Asp Pro Gln  
 755 760 765

Ile His Thr Pro Asp Gly Lys Gly Phe Gly Leu Gly Asn Leu Gly Lys  
 770 775 780

Ala Gly Ile Asn Lys Phe Ile Thr Thr His Lys Cys Asn Ala Val Cys  
 785 790 795 800

Ala Leu Leu Asp Leu Asp Val Lys Leu Gly Gly Val Leu Ser Gly Asn  
 805 810 815

Asn Lys Lys Gln Leu Gln Gln Gly Thr Met Val Met Pro Asp Ile Leu  
 820 825 830

Pro Glu Leu Met Pro Ser Asp Asn Thr Ile Lys Val Gly Ala Lys Gln  
 835 840 845

Leu Pro Lys Ala Glu Phe Ser Lys Lys Asp Leu Lys Cys Val Ser Thr  
 850 855 860

Ile Gln Ser Phe Arg Glu Arg Val Asn Ser Ile Ala Phe Phe Asp Asn  
 865 870 875 880

Gln Lys Leu Leu Cys Ala Gly Tyr Gly Asp Gly Thr Tyr Arg Val Phe  
 885 890 895

Asp Val Asn Asp Asn Trp Lys Cys Leu Tyr Thr Val Asn Gly His Arg  
 900 905 910

Lys Ser Ile Glu Ser Ile Ala Cys Asn Ser Asn Tyr Ile Phe Thr Ser  
 915 920 925

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Ser Pro Asp Asn Thr Ile Lys Val His Ile Ile Arg Ser Gly Asn Thr  
 930 935 940  
 Lys Cys Ile Glu Thr Leu Val Gly His Thr Gly Glu Val Asn Cys Val  
 945 950 955 960  
 Val Ala Asn Glu Lys Tyr Leu Phe Ser Cys Ser Tyr Asp Lys Thr Ile  
 965 970 975  
 Lys Val Trp Asp Leu Ser Thr Phe Lys Glu Ile Lys Ser Phe Glu Gly  
 980 985 990  
 Val His Thr Lys Tyr Ile Lys Thr Leu Ala Leu Ser Gly Arg Tyr Leu  
 995 1000 1005  
 Phe Ser Gly Gly Asn Asp Gln Ile Ile Tyr Val Trp Asp Thr Glu Thr  
 1010 1015 1020  
 Leu Ser Met Leu Phe Asn Met Gln Gly His Glu Asp Trp Val Leu Ser  
 1025 1030 1035 1040  
 Leu His Cys Thr Ala Ser Tyr Leu Phe Ser Thr Ser Lys Asp Asn Val  
 1045 1050 1055  
 Ile Lys Ile Trp Asp Leu Ser Asn Phe Ser Cys Ile Asp Thr Leu Lys  
 1060 1065 1070  
 Gly His Trp Asn Ser Val Ser Ser Cys Val Val Lys Asp Arg Tyr Leu  
 1075 1080 1085  
 Tyr Ser Gly Ser Glu Asp Asn Ser Ile Lys Val Trp Asp Leu Asp Thr  
 1090 1095 1100  
 Leu Glu Cys Val Tyr Thr Ile Pro Lys Ser His Ser Leu Gly Val Lys  
 1105 1110 1115 1120  
 Cys Leu Met Val Phe Asn Asn Gln Ile Ile Ser Ala Ala Phe Asp Gly  
 1125 1130 1135  
 Ser Ile Lys Val Trp Glu Trp Gln Ser Lys  
 1140 1145

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2237 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Dictyostelium discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATAAGAAGAT AGAAGATGAT ATTTAAAGTT TGGTTTTTCAT ATGAAGATGA GGAAGTGGAA	60
CTATCAGAAT TAACAAATGA TACAACAGTG TCAGCAATTA GAAAGATCTT ACATGAAGGT	120
AAAATATTTA GATTTCCATA TGGTACATCT CAAACAGACT TGCAAATTGG AAAGATGTTA	180
CCATCTGGTA GTGGTGGAGG TGCAACTGCA GACAGCAAAT TTGAGAAGTT TAAAGCACGT	240
AATACATTAG CAGATATTCA ATATAAAGTT GGTGATACAT TATATGTTAG AGTTAAAAAA	300
AGTAAACCAA CAAATGATTC ATTATTACCA ACATTAAATA TAGCATTTTT AGATGGATCA	360
GAACGTGCAA TTAAATGGGA ATATGACCCA TATACTACAA CTGCTCAATG GACCTGTACA	420
GCAACATTAG TCAAAGTTGA ACCAGTACCA TTTGCTGAAG GTGCATTTAG GAAAGCTTAT	480
CATACATTGG ATTTAAGTAA ATCTGGTGCA AGTGGAAGAT ATGTATCAAA GATTGGTAAA	540
AAACCAACAC CAAGACCATC ATATTTTGAA GATGTAAAGA TGCAAATGAT AGCAAAGAAA	600
TGGGCAGATA AATATAATTC ATTTAAACCT CCAAAAAAGA TTGAATTTTT ACAATCATGC	660
GTTTTAGAGT TTGTAGATAG AACATCATCA GATTTAATTT GTGGAGCAGA ACCATATGTA	720
GAAGGACAAT ATAGAAAGTA TAATAATAAT AGTGGATTCG TTAGTAATGA TGAAAGAAAT	780
ACACCACAAT CATTCTCTCA TTTCACATAT GAACATTCAA ATCATCAATT ATTGATTATA	840
GATATTCAAG GTGTTGGTGA TCACTATACA GACCCACAAA TTCATACCTA TGATGGTGTT	900
GGTTTTGGTA TTGGTAATTT GGGTCAAAAA GGTTTTGAAA AGTTTTTTAGA TACTCATAAA	960
TGTAATGCAA TTTGCCAATA TTAAATTTA CAATCAATTA ATCCAAAATC TGAAAAAAGT	1020
GATTGTGGTA CTGTACCAAG ACCAGATTTA ATTTCCCTG ATACATCTGA AAGAGATAAT	1080
AATAATAATA ATAATAATAA TAATAATAAT AATAATAATA ATAATAATAA TAATAGTAAT	1140
AATAATAATA ATAACAATAG TAGTATTTCA AAATCATTAG TTGAAATTTT AAGTGGTAGT	1200
AAAGAAAGAA ATGATAGAGA TTCGCCAAGT AGACAATTAT TTGTTTCAAA TGATGGTAAT	1260



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ACATTAAATA CAAATAAAGA GAGATCAAAA TCAAAATCAA TAGATTTAGA AAAACCAGAA	1320
ATTTTAATAA ATAATAAGAA AAAAGAGAGT ATAAATTTGG AAACGATAAA ATTAATTGAA	1380
ACTATTAAAG GATATCATGT TACAAGTCAT TTATGTATTT GTGATAATTT ATTATTTACA	1440
GGATGTTTCA ATAATTCAAT TAGAGTGTAT GATTATAAGA GTCAAAATAT GGAATGTGTT	1500
CAAACCTTGA AAGGTCATGA AGGTCCAGTT GAATCAATTT GTTATAATGA TCAATATTTG	1560
TTTAGTGGTT CATCAGATCA TTCAATTAAA GTTTGGGATT TAAAGAAATT AAGATGTATT	1620
TTTACTTTGG AGGGTCATGA TAAACCTGTC CATACGGTTC TATTGAATGA TAAATATTTG	1680
TTTAGTGGTT CCTCTGACAA AACTATCAAA GTTTGGGATT TGAAAACTTT GGAATGTAAA	1740
TATACCCTTG AAAGTCATGC CAGAGCCGTC AAAACACTTT GTATATCTGG TCAATATTTA	1800
TTTAGTGGTT CAAATGATAA AACTATCAAG GTTTGGGATT TGAAAACTTT TCGTTGTAAC	1860
TACACTCTAA AAGGTCATAC TAAATGGGTC ACCACTATCT GTATATTAGG TACCAATCTC	1920
TACAGTGGCT CCTATGATAA AACTATAAGA GTTTGGGAATT TAAAGAGTTT AGAATGTTCC	1980
GCTACTTTAA GAGGCCATGA TAGATGGGTT GAACATATGG TAATTTGTGA TAAATTATTA	2040
TTTACTGCTA GTGACGATAA TACAATTAAA ATTTGGGATT TAGAAACATT AAGATGTAAT	2100
ACAACTTTGG AAGGACATAA TGCAACCGTT CAATGTTTAG CAGTTTGGGA AGATAAAAAA	2160
TGTGTTATTA GTTGTAGTCA TGATCAAAGT ATTAGAGTTT GGGGTTGGAA TTAATTTAAA	2220
ATAAAAAAAA AAAACAT	2237

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 732 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Dictyostelium discoideum

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Ile	Phe	Lys	Val	Trp	Phe	Ser	Tyr	Glu	Asp	Glu	Glu	Val	Glu	Leu	1	5	10	15
Ser	Glu	Leu	Thr	Asn	Asp	Thr	Thr	Val	Ser	Ala	Ile	Arg	Lys	Ile	Leu	20	25	30	
His	Glu	Gly	Lys	Ile	Phe	Arg	Phe	Pro	Tyr	Gly	Thr	Ser	Gln	Thr	Asp	35	40	45	
Leu	Gln	Ile	Gly	Lys	Met	Leu	Pro	Ser	Gly	Ser	Gly	Gly	Gly	Ala	Thr	50	55	60	
Ala	Asp	Ser	Lys	Phe	Glu	Lys	Phe	Lys	Ala	Arg	Asn	Thr	Leu	Ala	Asp	65	70	75	80
Ile	Gln	Tyr	Lys	Val	Gly	Asp	Thr	Leu	Tyr	Val	Arg	Val	Lys	Lys	Ser	85	90	95	
Lys	Pro	Thr	Asn	Asp	Ser	Leu	Leu	Pro	Thr	Leu	Asn	Ile	Ala	Phe	Leu	100	105	110	
Asp	Gly	Ser	Glu	Arg	Ala	Ile	Lys	Trp	Glu	Tyr	Asp	Pro	Tyr	Thr	Thr	115	120	125	
Thr	Ala	Gln	Trp	Thr	Cys	Thr	Ala	Thr	Leu	Val	Lys	Val	Glu	Pro	Val	130	135	140	
Pro	Phe	Ala	Glu	Gly	Ala	Phe	Arg	Lys	Ala	Tyr	His	Thr	Leu	Asp	Leu	145	150	155	160
Ser	Lys	Ser	Gly	Ala	Ser	Gly	Arg	Tyr	Val	Ser	Lys	Ile	Gly	Lys	Lys	165	170	175	
Pro	Thr	Pro	Arg	Pro	Ser	Tyr	Phe	Glu	Asp	Val	Lys	Met	Gln	Met	Ile	180	185	190	
Ala	Lys	Lys	Trp	Ala	Asp	Lys	Tyr	Asn	Ser	Phe	Lys	Pro	Pro	Lys	Lys	195	200	205	
Ile	Glu	Phe	Leu	Gln	Ser	Cys	Val	Leu	Glu	Phe	Val	Asp	Arg	Thr	Ser	210	215	220	
Ser	Asp	Leu	Ile	Cys	Gly	Ala	Glu	Pro	Tyr	Val	Glu	Gly	Gln	Tyr	Arg	225	230	235	240
Lys	Tyr	Asn	Asn	Asn	Ser	Gly	Phe	Val	Ser	Asn	Asp	Glu	Arg	Asn	Thr	245	250	255	
Pro	Gln	Ser	Phe	Ser	His	Phe	Thr	Tyr	Glu	His	Ser	Asn	His	Gln	Leu				

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260					265					270					
Leu	Ile	Ile	Asp	Ile	Gln	Gly	Val	Gly	Asp	His	Tyr	Thr	Asp	Pro	Gln
		275					280						285		
Ile	His	Thr	Tyr	Asp	Gly	Val	Gly	Phe	Gly	Ile	Gly	Asn	Leu	Gly	Gln
	290					295					300				
Lys	Gly	Phe	Glu	Lys	Phe	Leu	Asp	Thr	His	Lys	Cys	Asn	Ala	Ile	Cys
305					310					315					320
Gln	Tyr	Leu	Asn	Leu	Gln	Ser	Ile	Asn	Pro	Lys	Ser	Glu	Lys	Ser	Asp
			325						330					335	
Cys	Gly	Thr	Val	Pro	Arg	Pro	Asp	Leu	Ile	Phe	Pro	Asp	Thr	Ser	Glu
			340					345					350		
Arg	Asp	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Asn
		355					360					365			
Asn	Asn	Asn	Asn	Asn	Ser	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Asn	Ser	Ser
	370					375					380				Ile
Ser	Lys	Ser	Leu	Val	Glu	Ile	Ser	Ser	Gly	Ser	Lys	Glu	Arg	Asn	Asp
385					390					395					400
Arg	Asp	Ser	Pro	Ser	Arg	Gln	Leu	Phe	Val	Ser	Asn	Asp	Gly	Asn	Thr
				405					410					415	
Leu	Asn	Thr	Asn	Lys	Glu	Arg	Ser	Lys	Ser	Lys	Ser	Ile	Asp	Leu	Glu
			420					425					430		
Lys	Pro	Glu	Ile	Leu	Ile	Asn	Asn	Lys	Lys	Lys	Glu	Ser	Ile	Asn	Leu
		435					440					445			
Glu	Thr	Ile	Lys	Leu	Ile	Glu	Thr	Ile	Lys	Gly	Tyr	His	Val	Thr	Ser
	450					455					460				
His	Leu	Cys	Ile	Cys	Asp	Asn	Leu	Leu	Phe	Thr	Gly	Cys	Ser	Asp	Asn
465					470					475					480
Ser	Ile	Arg	Val	Tyr	Asp	Tyr	Lys	Ser	Gln	Asn	Met	Glu	Cys	Val	Gln
				485					490					495	
Thr	Leu	Lys	Gly	His	Glu	Gly	Pro	Val	Glu	Ser	Ile	Cys	Tyr	Asn	Asp
			500					505					510		
Gln	Tyr	Leu	Phe	Ser	Gly	Ser	Ser	Asp	His	Ser	Ile	Lys	Val	Trp	Asp
		515					520					525			
Leu	Lys	Lys	Leu	Arg	Cys	Ile	Phe	Thr	Leu	Glu	Gly	His	Asp	Lys	Pro

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530		535		540
Val His Thr Val Leu Leu Asn Asp Lys Tyr Leu Phe Ser Gly Ser Ser				
545		550		555 560
Asp Lys Thr Ile Lys Val Trp Asp Leu Lys Thr Leu Glu Cys Lys Tyr				
	565		570	575
Thr Leu Glu Ser His Ala Arg Ala Val Lys Thr Leu Cys Ile Ser Gly				
	580		585	590
Gln Tyr Leu Phe Ser Gly Ser Asn Asp Lys Thr Ile Lys Val Trp Asp				
	595		600	605
Leu Lys Thr Phe Arg Cys Asn Tyr Thr Leu Lys Gly His Thr Lys Trp				
	610		615	620
Val Thr Thr Ile Cys Ile Leu Gly Thr Asn Leu Tyr Ser Gly Ser Tyr				
	625		630	635 640
Asp Lys Thr Ile Arg Val Trp Asn Leu Lys Ser Leu Glu Cys Ser Ala				
	645		650	655
Thr Leu Arg Gly His Asp Arg Trp Val Glu His Met Val Ile Cys Asp				
	660		665	670
Lys Leu Leu Phe Thr Ala Ser Asp Asp Asn Thr Ile Lys Ile Trp Asp				
	675		680	685
Leu Glu Thr Leu Arg Cys Asn Thr Thr Leu Glu Gly His Asn Ala Thr				
	690		695	700
Val Gln Cys Leu Ala Val Trp Glu Asp Lys Lys Cys Val Ile Ser Cys				
	705		710	715 720
Ser His Asp Gln Ser Ile Arg Val Trp Gly Trp Asn				
	725		730	

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2307 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: *C. elegans*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGACGATCG ACACAACAAA TGAGAGCGAC AATAGTCCAA CTAATCACC AGGATTGGAG	60
GCCTCGGCTC GGACATTCTC GCTCAATGCG TCAAAAATGG TTCGGATAAC CGACGACTAC	120
GCAGATGAAG TGTTCAATTGA ACAGAATGAT GTCGTTATCG AGAAGCCTCG TATGGATCCT	180
CTCCACGTTA GAAAACTTAT GGAGACATGG CGCAAGGCTG CTCGCCGAGC AAGAACAAAC	240
TATATAGATC CATGGGATGA GTTCAACATC CACGAGTATC CAGTACAACG AGCTAAACGA	300
TATAGGTATT CTGCAATCAG AAAGCAATGG ACAGAGGATA TAGTCGATGT GAGACTTCAT	360
CCGGACAGTT TTGCACGTGG AGCCATGCGA GAATGCTACC GACTCAAAAA GTGCTCCAAG	420
CACGGAACAA GTCAAGATTG GAGCAGCAAC TATGTCGCAA AAAGATACAT TTGTCAAGTC	480
GATCGTAGAG TTCTTTTTCGA TGATGTCAGA CTTCAGATGG ATGCCAAATT ATGGGCTGAA	540
GAATATAATC GGTATAATCC ACCGAAGAAA ATTGATATTG TTCAAATGTG TGTCATTGAG	600
ATGATTGATG TAAAAGGTTT TCCACTCTAT CATTTGGAGC ATTTTCATCGA GGGAAAATAT	660
ATAAAATACA ATTCAAATC AGGATTTGTA TCAAATGCAG CTCGTCTTAC ACCACAAGCA	720
TTTTCTCACT TCACCTTCGA ACGTTCTGGT CATCAAATGA TGGTTGTCGA TATTCAAGGA	780
GTTGGTGATC TTTACACAGA TCCTCAGATT CATAAGTTG TGGGAAGTGA TTATGGAGAT	840
GGAAACCTCG GAACTCGTGG AATGGCTCTT TTCTTCCATT CACACAGATG TAACGATATT	900
TGTGAGACAA TGGATCTATC AAATTTTCGAA CTTTCGCCAC CTGAAATCGA GGCTACCGAA	960
GTTGCGATGG AAGTAGCTGC AAAGCAGAAA AAGTCATGCA TAGTTCCTCC AACTGTGTTC	1020
GAAGCAAGAA GAAATCGAAT TTCAAGTGAA TGTGTACATG TCGAGCATGG TATTTTCGATG	1080
GATCAATTGA GAAAAAGGAA GACGTTGAAT CAATCGTCAA CCGATTTGTC AGCAAAGAGT	1140
CACAACGAAG ACTGTGTATG TCCTGAGTGT ATTCCAGTTG TTGAGCAACT CTGTGAGCCT	1200
TGCTCCGAAG ATGAAGAGGA CGAAGAAGAA GACTATCCAA GAAGTGAAAA AAGTGGAAT	1260
AGTCAGAAAA GTCGACGTAG TAGAATGAGC ATTTCAACGA GATCTTCTGG CGATGAATCA	1320
GCATCTCGTC CTAGAAAATG CGGATTTGTA GATTTAAACT CACTTCGTCA GAGACATGAT	1380

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AGCTTCAGAA GTTCTGTTGG GACATATTCT ATGAATAGTT CTAGACAAAC CAGAGACACT	1440
GAAAAGGATG AATTCTGGAA GGTTCCTCGA AAACAATCAG TTCCAGCAAA CATTCTATCA	1500
CTTCAACTTC AACAAATGGC TGCTAACCTG GAAAATGATG AAGACGTACC ACAAGTCACC	1560
GGGCATCAGT TCTCTGTCCT CGGTCAGATT CATATTGATC TCTCACGATA TCATGAGCTC	1620
GGGCGGTTCG TAGAAGTTGA TTCAGAACAT AAGGAAATGC TTGAGGGAAG TGAAAATGAC	1680
GCTCGTGATC CAATCAAATA CGACAAGCAG TCTGCAATTT TCCATTTGGA TATCGCTCGG	1740
AAGTGTGGAA TCCTTGAGGC TGTGCTAACA TCGGCTCATA TTGTTCTCGG ATTACCACAT	1800
GAATTGTTGA AAGAAGTCAC CGTTGATGAT CTGTTTCCTA ATGGGTTTGG AGAACAGGAA	1860
AATGGAATTC GAGCTGATAA AGGACAAAAA CCTTGTGACC TAGAAGAGTT CGGCTCCGAT	1920
CTGATGGAAA TTGCTGCAGA GATGGGTGAT AAGGGTGCAA TGCTGTACAT GGCACACGCT	1980
TATGAAACTG GTCAGCATCT CGGACCGAAT CGAAGAACGG ATTATAAGAA ATCGATTGAT	2040
TGGTATCAAC GCGTCGTTGG ATTCCAAGAA GAAGAAGAAC TTGACTCTGA TTGTGGAAAA	2100
ACGACATTCT CCTCATTTGC TCCACTGACT CGTCACGAGA TTCTAGCCAA AATGGCTGAA	2160
ATGTACAAAG AGGGAGGTTA TGGCCTGAAT CAAGACTTCG AACGAGCATA TGGTCTATTC	2220
AATGAAGCTG CTGAAGCAGC AATGGAAGCA ATGAATGGAA AGCTCGCAAA TAAATACTAT	2280
GAAAAAGCGG AAATGTGTGG AGAATGA	2307

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 768 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (iii) HYPOTHETICAL: NO

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: C. elegans

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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Met Thr Ile Asp Thr Thr Asn Glu Ser Asp Asn Ser Pro Thr Asn Ser  
 1 5 10 15  
 Pro Gly Leu Glu Ala Ser Ala Arg Thr Phe Ser Leu Asn Ala Ser Lys  
 20 25 30  
 Met Val Arg Ile Thr Asp Asp Tyr Ala Asp Glu Val Phe Ile Glu Gln  
 35 40 45  
 Asn Asp Val Val Ile Glu Lys Pro Arg Met Asp Pro Leu His Val Arg  
 50 55 60  
 Lys Leu Met Glu Thr Trp Arg Lys Ala Ala Arg Arg Ala Arg Thr Asn  
 65 70 75 80  
 Tyr Ile Asp Pro Trp Asp Glu Phe Asn Ile His Glu Tyr Pro Val Gln  
 85 90 95  
 Arg Ala Lys Arg Tyr Arg Tyr Ser Ala Ile Arg Lys Gln Trp Thr Glu  
 100 105 110  
 Asp Ile Val Asp Val Arg Leu His Pro Asp Ser Phe Ala Arg Gly Ala  
 115 120 125  
 Met Arg Glu Cys Tyr Arg Leu Lys Lys Cys Ser Lys His Gly Thr Ser  
 130 135 140  
 Gln Asp Trp Ser Ser Asn Tyr Val Ala Lys Arg Tyr Ile Cys Gln Val  
 145 150 155 160  
 Asp Arg Arg Val Leu Phe Asp Asp Val Arg Leu Gln Met Asp Ala Lys  
 165 170 175  
 Leu Trp Ala Glu Glu Tyr Asn Arg Tyr Asn Pro Pro Lys Lys Ile Asp  
 180 185 190  
 Ile Val Gln Met Cys Val Ile Glu Met Ile Asp Val Lys Gly Ser Pro  
 195 200 205  
 Leu Tyr His Leu Glu His Phe Ile Glu Gly Lys Tyr Ile Lys Tyr Asn  
 210 215 220  
 Ser Asn Ser Gly Phe Val Ser Asn Ala Ala Arg Leu Thr Pro Gln Ala  
 225 230 235 240  
 Phe Ser His Phe Thr Phe Glu Arg Ser Gly His Gln Met Met Val Val  
 245 250 255  
 Asp Ile Gln Gly Val Gly Asp Leu Tyr Thr Asp Pro Gln Ile His Thr  
 260 265 270



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Val Val Gly Thr Asp Tyr Gly Asp Gly Asn Leu Gly Thr Arg Gly Met  
 275 280 285

Ala Leu Phe Phe His Ser His Arg Cys Asn Asp Ile Cys Glu Thr Met  
 290 295 300

Asp Leu Ser Asn Phe Glu Leu Ser Pro Pro Glu Ile Glu Ala Thr Glu  
 305 310 315 320

Val Ala Met Glu Val Ala Ala Lys Gln Lys Lys Ser Cys Ile Val Pro  
 325 330 335

Pro Thr Val Phe Glu Ala Arg Arg Asn Arg Ile Ser Ser Glu Cys Val  
 340 345 350

His Val Glu His Gly Ile Ser Met Asp Gln Leu Arg Lys Arg Lys Thr  
 355 360 365

Leu Asn Gln Ser Ser Thr Asp Leu Ser Ala Lys Ser His Asn Glu Asp  
 370 375 380

Cys Val Cys Pro Glu Cys Ile Pro Val Val Glu Gln Leu Cys Glu Pro  
 385 390 395 400

Cys Ser Glu Asp Glu Glu Asp Glu Glu Glu Asp Tyr Pro Arg Ser Glu  
 405 410 415

Lys Ser Gly Asn Ser Gln Lys Ser Arg Arg Ser Arg Met Ser Ile Ser  
 420 425 430

Thr Arg Ser Ser Gly Asp Glu Ser Ala Ser Arg Pro Arg Lys Cys Gly  
 435 440 445

Phe Val Asp Leu Asn Ser Leu Arg Gln Arg His Asp Ser Phe Arg Ser  
 450 455 460

Ser Val Gly Thr Tyr Ser Met Asn Ser Ser Arg Gln Thr Arg Asp Thr  
 465 470 475 480

Glu Lys Asp Glu Phe Trp Lys Val Leu Arg Lys Gln Ser Val Pro Ala  
 485 490 495

Asn Ile Leu Ser Leu Gln Leu Gln Gln Met Ala Ala Asn Leu Glu Asn  
 500 505 510

Asp Glu Asp Val Pro Gln Val Thr Gly His Gln Phe Ser Val Leu Gly  
 515 520 525

Gln Ile His Ile Asp Leu Ser Arg Tyr His Glu Leu Gly Arg Phe Val  
 530 535 540

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Glu Val Asp Ser Glu His Lys Glu Met Leu Glu Gly Ser Glu Asn Asp  
 545 550 555 560  
 Ala Arg Val Pro Ile Lys Tyr Asp Lys Gln Ser Ala Ile Phe His Leu  
 565 570 575  
 Asp Ile Ala Arg Lys Cys Gly Ile Leu Glu Ala Val Leu Thr Ser Ala  
 580 585 590  
 His Ile Val Leu Gly Leu Pro His Glu Leu Leu Lys Glu Val Thr Val  
 595 600 605  
 Asp Asp Leu Phe Pro Asn Gly Phe Gly Glu Gln Glu Asn Gly Ile Arg  
 610 615 620  
 Ala Asp Lys Gly Gln Lys Pro Cys Asp Leu Glu Glu Phe Gly Ser Asp  
 625 630 635 640  
 Leu Met Glu Ile Ala Ala Glu Met Gly Asp Lys Gly Ala Met Leu Tyr  
 645 650 655  
 Met Ala His Ala Tyr Glu Thr Gly Gln His Leu Gly Pro Asn Arg Arg  
 660 665 670  
 Thr Asp Tyr Lys Lys Ser Ile Asp Trp Tyr Gln Arg Val Val Gly Phe  
 675 680 685  
 Gln Glu Glu Glu Glu Leu Asp Ser Asp Cys Gly Lys Thr Thr Phe Ser  
 690 695 700  
 Ser Phe Ala Pro Leu Thr Arg His Glu Ile Leu Ala Lys Met Ala Glu  
 705 710 715 720  
 Met Tyr Lys Glu Gly Gly Tyr Gly Leu Asn Gln Asp Phe Glu Arg Ala  
 725 730 735  
 Tyr Gly Leu Phe Asn Glu Ala Ala Glu Ala Ala Met Glu Ala Met Asn  
 740 745 750  
 Gly Lys Leu Ala Asn Lys Tyr Tyr Glu Lys Ala Glu Met Cys Gly Glu  
 755 760 765

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2283 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *C. elegans*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGACGATCG ACACAACAAA TGAGAGCGAC AATAGTCCAA CTAACTCACC AGGATTGGAG	60
GCCTCGGCTC GGACATTCTC GCTCAATGCG TCAAAAATGG TTCGGATAAC CGACGACTAC	120
GCAGATGAAG TGTTTCATTGA ACAGAATGAT GTCGTTATCG AGAAGCCTCG TATGGATCCT	180
CTCCACGTTA GAAAACTTAT GGAGACATGG CGCAAGGCTG CTCGCCGAGC AAGAACAAAC	240
TATATAGATC CATGGGATGA GTTCAACATC CACGAGTATC CAGTACAACG AGCTAAACGA	300
TATAGGTATT CTGCAATCAG AAAGCAATGG ACAGAGGATA TAGTCGATGT GAGACTTCAT	360
CCGGACAGTT TTGCACGTGG AGCCATGCGA GAATGCTACC GACTCAAAAA GTGCTCCAAG	420
CACGGAACAA GTCAAGATTG GAGCAGCAAC TATGTCGCAA AAAGATACAT TTGTCAAGTC	480
GATCGTAGAG TTCTTTTCGA TGATGTCAGA CTTCAGATGG ATGCCAAATT ATGGGCTGAA	540
GAATATAATC GGTATAATCC ACCGAAGAAA ATTGATATTG TTCAAATGTG TGTCATTGAG	600
ATGATTGATG TAAAAGGTTC TCCACTCTAT CATTTGGAGC ATTTTCATCGA GGGAAAATAT	660
ATAAAATACA ATTCAAATC AGGATTTGTA TCAAATGCAG CTCGTCTTAC ACCACAAGCA	720
TTTTCTCACT TCACCTTCGA ACGTTCTGGT CATCAAATGA TGGTTGTCTGA TATTCAAGGA	780
GTTGGTGATC TTTACACAGA TCCTCAGATT CATAAGTTG TGGGAACTGA TTATGGAGAT	840
GGAAACCTCG GAACTCGTGG AATGGCTCTT TTCTTCCATT CACACAGATG TAACGATATT	900
TGTGAGACAA TGGATCTATC AAATTTTCGAA CTTTCGCCAC CTGAAATCGA GGCTACCGAA	960
GTTGCGATGG AAGTAGCTGC AAAGCAGAAA AAGTCATGCA TAGTTCCTCC AACTGTGTTC	1020
GAAGCAAGAA GAAATCGAAT TTCAAGTGAA TGTGTACATG TCGAGCATGG TATTTTCGATG	1080
GATCAATTGA GAAAAAGGAA GACGTTGAAT CAATCGTCAA CCGATTTGTC AGCAAAGAGT	1140
CACAACGAAG ACTGTGTATG TCCTGAGTGT ATTCCAGTTG TTGAGCAACT CTGTGAGCCT	1200

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TGCTCCGAAG ATGAAGAGGA CGAAGAAGAA GACTATCCAA GAAGTGAAAA AAGTGGAAAT	1260
AGTCAGAAAA GTCGACGTAG TAGAATGAGC ATTTCAACGA GATCTTCTGG CGATGAATCA	1320
GCATCTCGTC CTAGAAAATG CGGATTTGTA GATTTAAACT CACTTCGTCA GAGACATGAT	1380
AGCTTCAGAA GTTCTGTTGG GACATATTCT ATGAATAGTT CTAGACAAAC CAGAGACACT	1440
GAAAAGGATG AATTCTGGAA GGTTCCTCGA AAACAATCAG TTCCAGCAAA CATTCTATCA	1500
CTTCAACTTC AACAAATGGC TGCTAACCTG GAAAATGATG AAGACGTACC ACAAGTCACC	1560
GGGCATCAGT TCTCTGTCCT CGGTCAGATT CATATTGATC TCTCACGATA TCATGAGCTC	1620
GGGCGGTTCG TAGAAGTTGA TTCAGAACAT AAGGAAATGC TTGAGGGAAG TGAAAATGAC	1680
GCTCGTGTAC CAATCAAATA CGACAAGCAG TCTGCAATTT TCCATTTGGA TATCGCTCGG	1740
AAGTGTGGAA TCCTTGAGGC TGTGCTAACA TCGGCTCATA TTGTTCTCGG ATTACCACAT	1800
GAATTGTTGA AAGAAGTCAC CGTTGATGAT CTGTTTCCTA ATGGGTTTGG AGAACAGGAA	1860
AATGGAATTC GAGACCTAGA AGAGTTCGGC TCCGATCTGA TGGAAATTGC TGCAGAGATG	1920
GGTGATAAGG GTGCAATGCT GTACATGGCA CACGCTTATG AAACTGGTCA GCATCTCGGA	1980
CCGAATCGAA GAACGGATTA TAAGAAATCG ATTGATTGGT ATCAACGCGT CGTTGGATTC	2040
CAAGAAGAAG AAGAACTTGA CTCTGATTGT GGAAAAACGA CATTCTCCTC ATTTGCTCCA	2100
CTGACTCGTC ACGAGATTCT AGCCAAAATG GCTGAAATGT ACAAAGAGGG AGGTTATGGC	2160
CTGAATCAAG ACTTCGAACG AGCATATGGT CTATTCAATG AAGCTGCTGA AGCAGCAATG	2220
GAAGCAATGA ATGGAAAGCT CGCAAATAAA TACTATGAAA AAGCGGAAAT GTGTGGAGAA	2280
TGA	2283

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 760 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

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(A) ORGANISM: *C. elegans*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Thr Ile Asp Thr Thr Asn Glu Ser Asp Asn Ser Pro Thr Asn Ser
1           5           10           15

Pro Gly Leu Glu Ala Ser Ala Arg Thr Phe Ser Leu Asn Ala Ser Lys
          20           25           30

Met Val Arg Ile Thr Asp Asp Tyr Ala Asp Glu Val Phe Ile Glu Gln
          35           40           45

Asn Asp Val Val Ile Glu Lys Pro Arg Met Asp Pro Leu His Val Arg
          50           55           60

Lys Leu Met Glu Thr Trp Arg Lys Ala Ala Arg Arg Ala Arg Thr Asn
65           70           75           80

Tyr Ile Asp Pro Trp Asp Glu Phe Asn Ile His Glu Tyr Pro Val Gln
          85           90           95

Arg Ala Lys Arg Tyr Arg Tyr Ser Ala Ile Arg Lys Gln Trp Thr Glu
          100          105          110

Asp Ile Val Asp Val Arg Leu His Pro Asp Ser Phe Ala Arg Gly Ala
          115          120          125

Met Arg Glu Cys Tyr Arg Leu Lys Lys Cys Ser Lys His Gly Thr Ser
          130          135          140

Gln Asp Trp Ser Ser Asn Tyr Val Ala Lys Arg Tyr Ile Cys Gln Val
145          150          155          160

Asp Arg Arg Val Leu Phe Asp Asp Val Arg Leu Gln Met Asp Ala Lys
          165          170          175

Leu Trp Ala Glu Glu Tyr Asn Arg Tyr Asn Pro Pro Lys Lys Ile Asp
          180          185          190

Ile Val Gln Met Cys Val Ile Glu Met Ile Asp Val Lys Gly Ser Pro
          195          200          205

Leu Tyr His Leu Glu His Phe Ile Glu Gly Lys Tyr Ile Lys Tyr Asn
          210          215          220

Ser Asn Ser Gly Phe Val Ser Asn Ala Ala Arg Leu Thr Pro Gln Ala
225          230          235          240

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Phe	Ser	His	Phe	Thr	Phe	Glu	Arg	Ser	Gly	His	Gln	Met	Met	Val	Val	
				245					250					255		
Asp	Ile	Gln	Gly	Val	Gly	Asp	Leu	Tyr	Thr	Asp	Pro	Gln	Ile	His	Thr	
			260					265					270			
Val	Val	Gly	Thr	Asp	Tyr	Gly	Asp	Gly	Asn	Leu	Gly	Thr	Arg	Gly	Met	
		275					280					285				
Ala	Leu	Phe	Phe	His	Ser	His	Arg	Cys	Asn	Asp	Ile	Cys	Glu	Thr	Met	
	290					295					300					
Asp	Leu	Ser	Asn	Phe	Glu	Leu	Ser	Pro	Pro	Glu	Ile	Glu	Ala	Thr	Glu	
305					310					315					320	
Val	Ala	Met	Glu	Val	Ala	Ala	Lys	Gln	Lys	Lys	Ser	Cys	Ile	Val	Pro	
			325					330						335		
Pro	Thr	Val	Phe	Glu	Ala	Arg	Arg	Asn	Arg	Ile	Ser	Ser	Glu	Cys	Val	
			340					345					350			
His	Val	Glu	His	Gly	Ile	Ser	Met	Asp	Gln	Leu	Arg	Lys	Arg	Lys	Thr	
		355					360					365				
Leu	Asn	Gln	Ser	Ser	Thr	Asp	Leu	Ser	Ala	Lys	Ser	His	Asn	Glu	Asp	
	370					375					380					
Cys	Val	Cys	Pro	Glu	Cys	Ile	Pro	Val	Val	Glu	Gln	Leu	Cys	Glu	Pro	
385					390					395					400	
Cys	Ser	Glu	Asp	Glu	Glu	Asp	Glu	Glu	Glu	Asp	Tyr	Pro	Arg	Ser	Glu	
			405					410					415			
Lys	Ser	Gly	Asn	Ser	Gln	Lys	Ser	Arg	Arg	Ser	Arg	Met	Ser	Ile	Ser	
			420					425					430			
Thr	Arg	Ser	Ser	Gly	Asp	Glu	Ser	Ala	Ser	Arg	Pro	Arg	Lys	Cys	Gly	
		435				440						445				
Phe	Val	Asp	Leu	Asn	Ser	Leu	Arg	Gln	Arg	His	Asp	Ser	Phe	Arg	Ser	
	450					455					460					
Ser	Val	Gly	Thr	Tyr	Ser	Met	Asn	Ser	Ser	Arg	Gln	Thr	Arg	Asp	Thr	
465					470					475					480	
Glu	Lys	Asp	Glu	Phe	Trp	Lys	Val	Leu	Arg	Lys	Gln	Ser	Val	Pro	Ala	
			485					490						495		
Asn	Ile	Leu	Ser	Leu	Gln	Leu	Gln	Gln	Met	Ala	Ala	Asn	Leu	Glu	Asn	
			500					505					510			

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Asp Glu Asp Val Pro Gln Val Thr Gly His Gln Phe Ser Val Leu Gly  
 515 520 525  
 Gln Ile His Ile Asp Leu Ser Arg Tyr His Glu Leu Gly Arg Phe Val  
 530 535 540  
 Glu Val Asp Ser Glu His Lys Glu Met Leu Glu Gly Ser Glu Asn Asp  
 545 550 555 560  
 Ala Arg Val Pro Ile Lys Tyr Asp Lys Gln Ser Ala Ile Phe His Leu  
 565 570 575  
 Asp Ile Ala Arg Lys Cys Gly Ile Leu Glu Ala Val Leu Thr Ser Ala  
 580 585 590  
 His Ile Val Leu Gly Leu Pro His Glu Leu Leu Lys Glu Val Thr Val  
 595 600 605  
 Asp Asp Leu Phe Pro Asn Gly Phe Gly Glu Gln Glu Asn Gly Ile Arg  
 610 615 620  
 Asp Leu Glu Glu Phe Gly Ser Asp Leu Met Glu Ile Ala Ala Glu Met  
 625 630 635 640  
 Gly Asp Lys Gly Ala Met Leu Tyr Met Ala His Ala Tyr Glu Thr Gly  
 645 650 655  
 Gln His Leu Gly Pro Asn Arg Arg Thr Asp Tyr Lys Lys Ser Ile Asp  
 660 665 670  
 Trp Tyr Gln Arg Val Val Gly Phe Gln Glu Glu Glu Glu Leu Asp Ser  
 675 680 685  
 Asp Cys Gly Lys Thr Thr Phe Ser Ser Phe Ala Pro Leu Thr Arg His  
 690 695 700  
 Glu Ile Leu Ala Lys Met Ala Glu Met Tyr Lys Glu Gly Gly Tyr Gly  
 705 710 715 720  
 Leu Asn Gln Asp Phe Glu Arg Ala Tyr Gly Leu Phe Asn Glu Ala Ala  
 725 730 735  
 Glu Ala Ala Met Glu Ala Met Asn Gly Lys Leu Ala Asn Lys Tyr Tyr  
 740 745 750  
 Glu Lys Ala Glu Met Cys Gly Glu  
 755 760

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:



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- (A) LENGTH: 628 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Dictyostelium discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTATTGTATG TGTTTCAATT GAGAAGACTC CATTTGCAAA GGGTAGTTGT AGAACAGCAC	60
ATAAATTAAA GGATTGGAGT CAACCAGATC AAGGATTAGT TGGTAAATTT TCAACCAATA	120
AAAAGACGAC AAGAGATTCA TACTTTACAG ATGTATTGAT GCAAACATTT TGTGCTAAAT	180
GGGCAGAGAA ATTCAATGAA GCGAAACCAC CAAAACCAAT TACATTCTTA CCATCATACG	240
TTTACGAATT GATTGATCAT CCACCACCTT ATCCAGTTTG TGGTGGTGAA CCATTCATTG	300
AGGGAGATTA CAAGAAACAT AACAACAACA GTGGTTACGT TAGTAGTGAT GCTAGAAATA	360
CACCACAATC ATTCTCTCAT TTCTCATACG AACTCTCCAA TCATGAATTG TTGATCGTTG	420
ATATCCAAGG TGTCACGAT TTCTACACTG ATCCTCAAAT TCATACGAAA TCAGGCGAGG	480
GCTTTGGCGA GGGTAATTTG GGCGAGACGG GTTCCACAA ATTCCTTCAA ACACACAAGT	540
GTAATCCAGT TTGTGACTTT TTAAAGTTGA AACCAATCAA TCAATCAAAG AAAGCCCTCC	600
TAAGAGGTAC ATTACCCGTC GTACAATT	628

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 209 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

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(A) ORGANISM: Dictyostelium discoideum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ile	Val	Cys	Val	Ser	Ile	Glu	Lys	Thr	Pro	Phe	Ala	Lys	Gly	Ser	Cys	1	5	10	15
Arg	Thr	Ala	His	Lys	Leu	Lys	Asp	Trp	Ser	Gln	Pro	Asp	Gln	Gly	Leu	20	25	30	
Val	Gly	Lys	Phe	Ser	Thr	Asn	Lys	Lys	Thr	Thr	Arg	Asp	Ser	Tyr	Phe	35	40	45	
Thr	Asp	Val	Leu	Met	Gln	Thr	Phe	Cys	Ala	Lys	Trp	Ala	Glu	Lys	Phe	50	55	60	
Asn	Glu	Ala	Lys	Pro	Pro	Lys	Pro	Ile	Thr	Phe	Leu	Pro	Ser	Tyr	Val	65	70	75	80
Tyr	Glu	Leu	Ile	Asp	His	Pro	Pro	Pro	Tyr	Pro	Val	Cys	Gly	Gly	Glu	85	90	95	
Pro	Phe	Ile	Glu	Gly	Asp	Tyr	Lys	Lys	His	Asn	Asn	Asn	Ser	Gly	Tyr	100	105	110	
Val	Ser	Ser	Asp	Ala	Arg	Asn	Thr	Pro	Gln	Ser	Phe	Ser	His	Phe	Ser	115	120	125	
Tyr	Glu	Leu	Ser	Asn	His	Glu	Leu	Leu	Ile	Val	Asp	Ile	Gln	Gly	Val	130	135	140	
Asn	Asp	Phe	Tyr	Thr	Asp	Pro	Gln	Ile	His	Thr	Lys	Ser	Gly	Glu	Gly	145	150	155	160
Phe	Gly	Glu	Gly	Asn	Leu	Gly	Glu	Thr	Gly	Phe	His	Lys	Phe	Leu	Gln	165	170	175	
Thr	His	Lys	Cys	Asn	Pro	Val	Cys	Asp	Phe	Leu	Lys	Leu	Lys	Pro	Ile	180	185	190	
Asn	Gln	Ser	Lys	Lys	Ala	Leu	Leu	Arg	Gly	Thr	Leu	Pro	Val	Val	Gln	195	200	205	
Leu																			

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 238 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gly	Glu	Trp	Leu	Asp	Asp	Glu	Val	Leu	Ile	Lys	Met	Ala	Ser	Gln	Pro	1	5	10	15
Phe	Gly	Arg	Gly	Ala	Met	Arg	Glu	Cys	Phe	Arg	Thr	Lys	Lys	Leu	Ser	20	25	30	
Asn	Phe	Leu	His	Ala	Gln	Gln	Trp	Lys	Gly	Ala	Ser	Asn	Tyr	Val	Ala	35	40	45	
Lys	Arg	Tyr	Ile	Glu	Pro	Val	Asp	Arg	Asp	Val	Tyr	Phe	Glu	Asp	Val	50	55	60	
Arg	Leu	Gln	Met	Glu	Ala	Lys	Leu	Trp	Gly	Glu	Glu	Tyr	Asn	Arg	His	65	70	75	80
Lys	Pro	Pro	Lys	Gln	Val	Asp	Ile	Met	Gln	Met	Cys	Ile	Ile	Glu	Leu	85	90	95	
Lys	Asp	Arg	Pro	Gly	Lys	Pro	Leu	Phe	His	Leu	Glu	His	Tyr	Ile	Glu	100	105	110	
Gly	Lys	Tyr	Ile	Lys	Tyr	Asn	Ser	Asn	Ser	Gly	Phe	Val	Arg	Asp	Asp	115	120	125	
Asn	Ile	Arg	Leu	Thr	Pro	Gln	Ala	Phe	Ser	His	Phe	Thr	Phe	Glu	Arg	130	135	140	
Ser	Gly	His	Gln	Leu	Ile	Val	Val	Asp	Ile	Gln	Gly	Val	Gly	Asp	Leu	145	150	155	160
Tyr	Thr	Asp	Pro	Gln	Ile	His	Thr	Glu	Thr	Gly	Thr	Asp	Phe	Gly	Asp	165	170	175	
Gly	Asn	Leu	Gly	Val	Arg	Gly	Met	Ala	Leu	Phe	Phe	Tyr	Ser	His	Ala	180	185	190	

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Cys Asn Arg Ile Cys Glu Ser Met Gly Leu Ala Pro Phe Asp Leu Ser  
 195 200 205

Pro Arg Glu Arg Asp Ala Val Asn Gln Asn Thr Lys Leu Leu Gln Ser  
 210 215 220

Ala Lys Thr Ile Leu Arg Gly Thr Glu Glu Lys Cys Gly Ser  
 225 230 235

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 258 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: D. discoideum

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asn Lys Trp Ile Arg Leu Ser Met Lys Leu Lys Val Glu Arg Lys Pro  
 1 5 10 15

Phe Ala Glu Gly Ala Leu Arg Glu Ala Tyr His Thr Val Ser Leu Gly  
 20 25 30

Val Gly Thr Asp Glu Asn Tyr Pro Leu Gly Thr Thr Thr Lys Leu Phe  
 35 40 45

Pro Pro Ile Glu Met Ile Ser Pro Ile Ser Lys Asn Asn Glu Ala Met  
 50 55 60

Thr Gln Leu Lys Asn Gly Thr Lys Phe Val Leu Lys Leu Tyr Lys Lys  
 65 70 75 80

Glu Ala Glu Gln Gln Ala Ser Arg Glu Leu Tyr Phe Glu Asp Val Lys  
 85 90 95

Met Gln Met Val Cys Arg Asp Trp Gly Asn Lys Phe Asn Gln Lys Lys  
 100 105 110

Pro Pro Lys Lys Ile Glu Phe Leu Met Ser Trp Val Val Glu Leu Ile  
 115 120 125

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Asp Arg Ser Pro Ser Ser Asn Gly Gln Pro Ile Leu Cys Ser Ile Glu  
 130 135 140  
 Pro Leu Leu Val Gly Glu Phe Lys Lys Asn Asn Ser Asn Tyr Gly Ala  
 145 150 155 160  
 Val Leu Thr Asn Arg Ser Thr Pro Gln Ala Phe Ser His Phe Thr Tyr  
 165 170 175  
 Glu Leu Ser Asn Lys Gln Met Ile Val Val Asp Ile Gln Gly Val Asp  
 180 185 190  
 Asp Leu Tyr Thr Asp Pro Gln Ile His Thr Pro Asp Gly Lys Gly Phe  
 195 200 205  
 Gly Leu Gly Asn Leu Gly Lys Ala Gly Ile Asn Lys Phe Ile Thr Thr  
 210 215 220  
 His Lys Cys Asn Ala Val Cys Ala Leu Leu Asp Leu Asp Val Lys Leu  
 225 230 235 240  
 Gly Gly Val Leu Ser Gly Asn Asn Lys Lys Gln Leu Gln Gln Gly Thr  
 245 250 255  
 Met Val

## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 212 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: D. discoideum

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Gln Trp Thr Cys Thr Ala Thr Leu Val Lys Val Glu Pro Val Pro  
 1 5 10 15  
 Phe Ala Glu Gly Ala Phe Arg Lys Ala Tyr His Thr Leu Asp Leu Ser  
 20 25 30

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Lys Ser Gly Ala Ser Gly Arg Tyr Val Ser Lys Ile Gly Lys Lys Pro  
           35                                  40                                  45  
 Thr Pro Arg Pro Ser Tyr Phe Glu Asp Val Lys Met Gln Met Ile Ala  
           50                                  55                                  60  
 Lys Lys Trp Ala Asp Lys Tyr Asn Ser Phe Lys Pro Pro Lys Lys Ile  
   65                                  70                                  75                                  80  
 Glu Phe Leu Gln Ser Cys Val Leu Glu Phe Val Asp Arg Thr Ser Ser  
                                   85                                  90                                  95  
 Asp Leu Ile Cys Gly Ala Glu Pro Tyr Val Glu Gly Gln Tyr Arg Lys  
                                   100                                  105                                  110  
 Tyr Asn Asn Asn Ser Gly Phe Val Ser Asn Asp Glu Arg Asn Thr Pro  
                                   115                                  120                                  125  
 Gln Ser Phe Ser His Phe Thr Tyr Glu His Ser Asn His Gln Leu Leu  
           130                                  135                                  140  
 Ile Ile Asp Ile Gln Gly Val Gly Asp His Tyr Thr Asp Pro Gln Ile  
   145                                  150                                  155                                  160  
 His Thr Tyr Asp Gly Val Gly Phe Gly Ile Gly Asn Leu Gly Gln Lys  
                                   165                                  170                                  175  
 Gly Phe Glu Lys Phe Leu Asp Thr His Lys Cys Asn Ala Ile Cys Gln  
                                   180                                  185                                  190  
 Tyr Leu Asn Leu Gln Ser Ile Asn Pro Lys Ser Glu Lys Ser Asp Cys  
                                   195                                  200                                  205  
 Gly Thr Val Pro  
           210

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 233 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: C. elegans

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Lys	Gln	Trp	Thr	Glu	Asp	Ile	Val	Asp	Val	Arg	Leu	His	Pro	Asp	Ser	1	5	10	15
Phe	Ala	Arg	Gly	Ala	Met	Arg	Glu	Cys	Tyr	Arg	Leu	Lys	Lys	Cys	Ser	20	25	30	
Lys	His	Gly	Thr	Ser	Gln	Asp	Trp	Ser	Ser	Asn	Tyr	Val	Ala	Lys	Arg	35	40	45	
Tyr	Ile	Cys	Gln	Val	Asp	Arg	Arg	Val	Leu	Phe	Asp	Asp	Val	Arg	Leu	50	55	60	
Gln	Met	Asp	Ala	Lys	Leu	Trp	Ala	Glu	Glu	Tyr	Asn	Arg	Tyr	Asn	Pro	65	70	75	80
Pro	Lys	Lys	Ile	Asp	Ile	Val	Gln	Met	Cys	Val	Ile	Glu	Met	Ile	Asp	85	90	95	
Val	Lys	Gly	Ser	Pro	Leu	Tyr	His	Leu	Glu	His	Phe	Ile	Glu	Gly	Lys	100	105	110	
Tyr	Ile	Lys	Tyr	Asn	Ser	Asn	Ser	Gly	Phe	Val	Ser	Asn	Ala	Ala	Arg	115	120	125	
Leu	Thr	Pro	Gln	Ala	Phe	Ser	His	Phe	Thr	Phe	Glu	Arg	Ser	Gly	His	130	135	140	
Gln	Met	Met	Val	Val	Asp	Ile	Gln	Gly	Val	Gly	Asp	Leu	Tyr	Thr	Asp	145	150	155	160
Pro	Gln	Ile	His	Thr	Val	Val	Gly	Thr	Asp	Tyr	Gly	Asp	Gly	Asn	Leu	165	170	175	
Gly	Thr	Arg	Gly	Met	Ala	Leu	Phe	Phe	His	Ser	His	Arg	Cys	Asn	Asp	180	185	190	
Ile	Cys	Glu	Thr	Met	Asp	Leu	Ser	Asn	Phe	Glu	Leu	Ser	Pro	Pro	Glu	195	200	205	
Ile	Glu	Ala	Thr	Glu	Val	Ala	Met	Glu	Val	Ala	Ala	Lys	Gln	Lys	Lys	210	215	220	
Ser	Cys	Ile	Val	Pro	Pro	Thr	Val	Phe	225	230									

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:



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- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligonucleotide Primer D"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGATTGGAC TGGACAAGAA CCCCC

25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Arg	Lys	Lys	Phe	Gly	Glu	Ser	Glu	Lys	Thr	Lys	Thr	Lys	Glu	Phe	Leu
1				5				10					15		

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Leu	Thr	Pro	Gln	Ala	Phe	Ser	His	Phe	Thr	Phe	Glu	Arg
1				5					10			

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Leu	Ala	Asn	Xaa	Tyr	Tyr	Glu	Lys	Ala	Glu
1				5					10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligonucleotides"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CANGCNTTNN NNCANTTNAC NTTNGANNG

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(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligonucleotides"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCNGCNTTNT CNTANTANTT NTTNGC

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(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligonucleotides"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TACAATCAGC TGATGACCAG AACGCTC

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